ROBUST SUMMARY OF INFORMATION ON

Substance:

CRUDE OIL

CAS No.

8002-05-9

OPPT CBIC

Summary prepared by:

American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)

A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.

Regulatory Toxicology and Pharmacology 25, 1-5.

1. General Information

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1.1.1 GENERAL SUBSTANCE INFORMATION

: Petroleum product Substance type

Physical status : Liquid

: The CAS definition for Petroleum (Crude oil) is: Remark

> "A complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen and sulfur compounds. This category encompasses light, medium, and heavy petroleums, as well as the oils extracted from tar sands".

Hydrocarbonaceous materials requiring major chemical changes for their recovery or conversion to petroleum refinery feedstocks such as crude shale oils, upgrade shale oils and liquid coal fuels are not included in this definition.

Crude oil contains hydrocarbons in the carbon number range from C1 to C60+. It also contains organometallic complexes, notably of sulfur and vanadium, and dissolved gases such as hydrogen sulfide. Crude oils range from thin, light colored oils consisting mainly of gasolinequality stock to heavy, thick tar-like materials.

An "average crude oil has the following general composition:

Carbon	84%
Hydrogen	14%
Sulfur	1-3%
Nitrogen	1%
Oxygen	1%
Minerals and salts	0.1%

The chemical composition of crude oils can vary tremendously from different producing regions and even from within a particular formation.

Examples of compositions of various whole crudes are shown in the following table

Crude source
Paraffins
% vol

Paraffins % vol.	Naphthenes % vol	Aromatics % vol	Sulfur % wt	API gravity <u>(°API)</u>
<u>Light crudes</u> Saudi light				
63	18	19	2.0	34
South Louisian	na			
79	45	19	0	35
Nigerian light				
37	54	9	0.1	36
North sea Bre	nt			
50	34	16	0.4	37

47 Lost Hills	34	19	0.4	37
Non-Aromatic	s 50%	50	0.9	
Heavy crudes	į			
Prudhoe Bay 27	36	28	0.9	28
Saudi Heavy 60	20	15	2.1	28
Venezuela He	•	12	2.2	24
35 Belridge Heav		12	2.3	24
Non-aromatic	s 37%	63	1.1	-
Mid-range cru	<u>ides</u>			
Kuwait 63	20	24	2.4	31
Venezuela Lig 52	ght 34	14	1.5	30
USA West Te		14	1.5	30

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Crude oils may be categorized in either of several different ways e.g.

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Paraffinic vs naphthenic.

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Crude oils contain both paraffinic and naphthenic hydrocarbons but if there is a preponderance of paraffinic hydrocarbons present, the crude oil is referred to as a paraffinic crude. These crudes would be rich in straight and branched chain paraffins. Conversely a crude in which naphthenic hydrocarbons are predominant is referred to as a naphthenic crude. These crudes contain mainly naphthenic and aromatic hydrocarbons

Sweet vs sour

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1. General Information

Crude oils may be referred to as either sweet or sour depending on the level of hydrogen sulfide present. A sweet crude has very little H_2S whereas a sour crude has larger quantities of H_2S present.

Light vs heavy

Crude oils may be divided into Light and Heavy crudes on the basis of their gravity.

The API gravity is determined as:

$$^{\circ}API = \frac{141.5}{\text{Specific gravity}} - 131.5$$

Crude oils with gravity > 33°API are considered as light crudes. Such crudes with a high percentage composition of hydrogen are usually more suitable for processing for gasoline production. Heavy crudes, ie those with gravity < 28°API tend to contain more asphaltenes and are usually rich in aromatics. These heavy crudes require more steps in their processing.

Information in this robust summary is presented for light and heavy crudes since this categorization distinguishes between crudes with a high paraffinic content (Light crudes) and those with a high aromatic/naphthenic content (Heavy crudes). This represents the extremes of the ranges of crudes available.

1. General Information

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1.13 REVIEWS

Memo : IARC Review

Remark : IARC reviewed the evidence for carcinogenicity of crude oil to man and

animals and published the result in 1989.

IARC concluded that:

There is inadequate evidence for the carcinogenicity in humans of

crude oil

There is limited evidence for the carcinogenicity in experimental

animals of crude oil

The overall evaluation was

Crude oil is not classifiable as to its carcinogenicity to humans

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2.1 MELTING POINT

Value : -30 - 30 °C

Sublimation: no

Method : ASTM D97 GLP : no data

Remark: The figures quoted are a typical range for the drop point as measured by a

standard oil industry procedure. For some low wax crudes, pour points

below -30 °C are obtained.

Reliability : (1) valid without restriction

(1)(16)

2.2 BOILING POINT

Value : -1 - 565 °C at 1013 hPa

Decomposition : yes

Method : ASTM D86 GLP : no data

Remark: The method measures the distillation range of petroleum products. The

figures quoted represent the approximate range for crude oils and are based on (a) the boiling point of n-butane for the lower value and (b) an upper limit quoted by Speight. In practice, atmospheric distillation of crude oil is not practiced above 275-300 °C, to avoid thermal decomposition of the residue. The residue is normally vacuum distilled in a subsequent

operation.

Reliability : (1) valid without restriction

(1) (16) (77)

2.4 VAPOUR PRESSURE

GLP : no data Test substance : Crude oil

Remark: Vapor pressure measurements were provided for 10 petroleum crude oils

originating from various locations throughout the world. The source of these data are from Environment Canada Environmental Technology Center, a government-maintained database (Jokuty et al., 2002). Data cited by OGJ may be considered a secondary source, but the data cited by ESD and EETD were measured data sponsored by those agencies using a standardized method for measuring vapor pressure of petroleum products¹. As such, these 10 measurements provide a body of data adequate for use in the U.S. EPA HPV program and specific for the physical/chemical

endpoint of vapor pressure.

(1) ASTM D323, Standard Test Method for vapor Pressure of Petroleum

Products (Reid Method)

Result: Vapor Pressure, kPa See the following table and Remarks section

Temperature °C Not stated Decomposition Not stated

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Vapor Pressures, kPa:

		Reid Vapor	
Product		Pressure	
Name	Origin	Value, kPa	Reference
Alaska North Slope	Alaska USA	19	ESD 91
Arabian Medium	Saudi Arabia	22.1	OGJ 99
Alif	Yemen	45	OGJ 99
Amna	Libya	27	OGJ 99
Ashtart	Tunisia	13	OGJ 99
Atkinson			
Beaufo	ort Sea, Canada	6	ESD 91
Alberta Sweet Mixed I	Blend		
Alberta	a, Canada	19	EETD 84
Abu Al Bu Khoush	United Arab Emirates	24	OGJ 99
Beryl	North Sea, UK	36	OGJ 99
Bombay High	India	33	OGJ 99

Reliability : (2) valid with restrictions

Data obtained from a government (Environment Canada) database who sponsored the vapor pressure measurements cited by ESD and EETD.

(20) (21) (39) (74)

2.5 PARTITION COEFFICIENT

Log pow : 2 - 6 Method : Calculated

Remark: The calculation was done by the CLOGP Version 3.5 program (calculation

of Log partition coefficient octanol/water). The figures represent the spread of calculated and/or measured values for typical hydrocarbon components of crude oil. Calculated values for higher molecular weight hydrocarbons will be above 6, but such values are notional, since no correlation has been

established between calculated and experimental values.

Reliability : (1) valid without restriction

(16)(45)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water

Method : Preparation of water soluble fraction

Year : 1990 GLP : no Test substance : Crude oil

Method : Individual saturated crude oil solutions were prepared by adding

approximately 10 ml of the respective oil to 50-100 ml of water in 125 ml separatory funnels. Funnels were gently shaken for at least 48 hrs either with a magnetic stir bar or with a wrist action shaker, then placed in a temperature bath for at least 48 hr prior to analysis. Solubility at 5, 20 and 22 (±2C) °C was determined in both double distilled water and salt water (3% NaCl). The effect of water to oil ratio on the solubility of crude oil components was determined by injecting oil into sealed vials completely filled with water, and mixed at low speed for an equilibration period of 20 days. Analysis was determined by purge-and-trap/GC; solid sorbent

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Result

extraction with HPLC and fluorescence analysis.

: Fluorescence/HPLC analysis was inadequate in quantification of hydrocarbon composition.

Purge and trap/GC analysis results based on total benzene, toluene, ethyl benzene+xylenes (combined concentration) and naphthalenes (BTE+XN as mg/l) are reported along with viscosity (vis=c.p @ 20C) and density (d=g/cm³ @ 20°C) for each oil at respective temperatures (°C) in distilled and saltwater (22° reported as ± 2°C)

and saltwater (22° reported as	•	
Oil	BTE+XN (mg/l Distilled water) Saltwater ———
Norman Wells 4.41 vis; 0.832 d		
	30 (5) 29-33 (20)	25.5 (5)
	31.8-33.5 (22)	20 (22)
4.7 vis; 0.84 d	33 (20)	14.8 (5)
Alberta: 4.98 vis; 0.835 d	25.02(22)	
Swan Hills: (no vis. density)	35.1 (22)	
Prudhoe Bay: 34.9 vis; 0.901 c	129.01 (22)	
Lago Medio: 41.1 vis; 0.872 d Kopanoar: 17.5 vis; 0.9 d Murban: 4.37 vis; 0.824 d	23.66-25.5 (22) 10.42 (22) 28.62 (22)	16.47 (22)
Hibernia B 33.2 vis; 0.837d Hibernia J (no vis., density) Mobil A (no vis., density) Mobil B (no vis., density)	29.6 (22) 58 (22)	16.92 (22) 7.75 (22)

Results for oil-water ratio testing were not quantified, but general observations were stated as follows: concentrations of the water soluble fractions decreases as the water-to oil ratio increases and the composition of the water soluble fraction changes as the ratio changes. At low water to oil ratios, the WSF is composed predominantly (80%) of BTEX. As the water to oil ratio increases, these compounds account for a smaller proportion of the dissolved compounds. At a water/oil ratio of 10000, these compounds account for only 15-30% of the total WSF.

Conclusion

Limited detail is provided for exact amounts of crude oil used for preparing aqueous solutions, nor is there any information regarding the composition of each of the crude oils tested, either as hydrocarbon type or inorganic components (such as sulfur). Also, no information on the GC calibration standard composition used to identify and quantify soluble components in the equilibrated aqueous -oil solutions is provided. Individual components of complex petroleum substances have specific and differing solubilities. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble

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components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that

is a non-linear function of the amount added.

Reliability (2) valid with restrictions

(14) (15) (18) (19) (47) (75)

2.14 ADDITIONAL REMARKS

Memo : CONCAWE

Remark : Petroleum is a natural organic material consisting mainly of hydrocarbons.

> It occurs in both the gaseous and liquid states in geological traps. The liquid phase, after being freed from dissolved gas and any associated salt water, is known as crude oil. All the information presented in this Data Set

relates to crude oil.

Crude oil is not a uniform substance since its physical and chemical properties vary from oilfield to oilfield and can even vary within wells at the same oilfield. At one extreme, crude oil is a light, mobile, straw-colored liquid containing a large proportion of hydrocarbons which are readily distilled at atmospheric pressure. At the other extreme, crude oil is a highly viscous, semi-solid, black substance from which little can be distilled at atmospheric pressure before thermal decomposition occurs.

The most consistent property of crude oils is their relatively small range of elemental composition, as the ranges in the following table show:

<u>element</u>	composition (wt%)
carbon	83.9 - 86.8
hydrogen	11.0 - 14.0
sulfur	0.06 - 8.0
nitrogen	0.02 - 1.7
oxygen	0.08 - 1.82
metals	0.00 - 0.14

Crude oils are normally characterized in terms of three properties; density, viscosity and sulfur content. Crude oils are identified as either light (specific gravity <0.82), or medium (specific gravity 0.82 to 0.97), or heavy (specific gravity > 0.97). The viscosity is an expression of the mobility of the crude oil. The sulfur content has a marked influence on the refinery procedures to which the crude oil, and in particular its derivatives, will be subjected in order to produce acceptable products.

Crude oils are also characterized in terms of their chemical composition. specifically on the predominance of the hydrocarbon types that are present. Modern practice tends to recognize two main types of crude, namely paraffinic and naphthenic. Paraffinic crude oils are rich in straight-chain and branched-chain alkanes, whereas in naphthenic crudes the main constituents are cycloparaffins and aromatic hydrocarbons. However, this is a simplified picture, as many crude oils fall between or outside these two

types.

The technical information has been compiled by the Oil Companies'

European Organization for Environmental Health Protection (CONCAWE), based at Madouplein-1, B-1210 Brussel, Belgium, and this organization

holds copies of the reference articles cited in this data set

Reliability (2) valid with restrictions

Source

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3.1.1 PHOTODEGRADATION

Type : air Sun light Light source

INDIRECT PHOTOLYSIS

Sensitizer OH

Conc. of sensitizer 1000000 molecule/cm3

Method Calculated according to Atkinson, 1990

Remark : Atkinson gives data which enables half lives to be calculated for the

> degradation of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight. Values for typical

hydrocarbon constituents of crude oils are as follows:

Constituent Half-life (days) benzene 6.5 n-butane 3.2 1.4 n-hexane toluene 1.3 cyclohexane 1.1 n-decane 0.69 n-tetradecane 0.42 0.37 naphthalene

Hydrocarbons of carbon number greater than C20 will have little or no

tendency to partition to air (see Sub-chapter3.2.2).

: (2) valid with restrictions Reliability

(3)(16)

3.1.2 STABILITY IN WATER

GLP : no

Remark : Hydrolysis of an organic chemical is the transformation process in which a

> water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters and sulfonic acid esters. The chemical components found in the materials that comprise the crude oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that

hydrolyze.

Reliability : (1) valid without restriction

(36)

3.3.2 DISTRIBUTION

air - biota - sediment(s) - soil - water Media Method Calculation according Mackay, Level I

Year 1981

Remark : Distribution has been calculated according to Mackay Level I using the

> parameters defined in a paper by van der Zandt and van Leeuwen. The lower molecular weight components will mainly partition to air, with a

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maximum of about 1% of mono-aromatic hydrocarbons partitioning to water. As the molecular weights increase, there is less tendency for the hydrocarbons to partition to air, the environmental distribution being preferentially to soil and sediment. Collectively, the wide molecular weight range of the hydrocarbons in crude oil will mean that at equilibrium, distribution will be mainly to air and soil, with much less than 1.0% being present in water.

Result

: Results for percent distribution of typical hydrocarbons found in crude oils are shown in tabular form as follows:

<u>air</u>	water	soil	sediment	susp.matter	biota
propar					
100.0		0.0	0.0	0.0	0.0
n-buta	_				
100.0		0.0	0.0	0.0	0.0
n-hexa	-				
100.0		0.0	0.0	0.0	0.0
n-octa	-	0.4		0.0	0.0
99.9	0.0	0.1	0.0	0.0	0.0
n-deca	_	0.0	0.0	0.0	0.0
	0.0	0.9	0.0	0.0	0.0
	decane	22.0	0.5	0.0	0.0
76.7 n-eicos	0.0	22.8	0.5	0.0	0.0
0.0	0.0	97.7	2.2	0.1	0.0
cycloh		91.1	2.2	0.1	0.0
99.9	0.0	0.1	0.0	0.0	0.0
benzer		0.1	0.0	0.0	0.0
98.8	_	0.1	0.0	0.0	0.0
toluene		0.1	0.0	0.0	0.0
98.4		0.6	0.0	0.0	0.0
p-xyler					
97.8		1.2	0.0	0.0	0.0
o-xyler	ne				
-	1.2	1.4	0.0	0.0	0.0
ethylbe	enzene				
98.2	8.0	1.0	0.0	0.0	0.0
n-butyl	lbenzen	е			
91.0	0.4	8.5	0.2	0.0	0.0

Reliability

: (1) valid without restriction

(16)(46)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5

Method : Theoretical Oxygen Demand (TOD) was calculated

Year : 1971 GLP : no data

Method : Tests were run at 30 °C in fresh water using a respirometric procedure with

crude oil at 50 and 70 mg/l. Prior to the test, the crude was "topped" at up to 100 °C to remove light ends, which comprised about 10% of the material

Result : The BOD/TOD ratio after 10 days was 0.3 at 50 mg/l and 0.04 at 70 mg/l.

With the addition of nutrients, using ammonia at up to 4.6 mg/l of nitrogen and phosphate at up to 15 mg/l of phosphorus, the BOD/TOD ratio increased to a maximum of 0.3 after 5 days, and 0.34 after 10 days. In further studies, run at 10 °C in sea water in the presence of nutrients, the

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degradation rate was found to be lowered by a factor of 2 to 3 below that at 30 °C. The reference quotes the BOD5/TOD ratio, but does not give the

individual values for BOD5 and TOD.

Reliability : (2) valid with restrictions

(7)(16)

3.8 ADDITIONAL REMARKS

Memo : General

Remark

The world production of crude oil per year is of the order of 3 billion tonnes per year, of which about half is transported by sea. In his 1989 publication, Clark states that the best estimate of petroleum hydrocarbons entering the sea per year is about 3 million tonnes, of which about one million tonnes is attributable to crude oil. Such pollution arises from the cleaning of oil tanker compartments, offshore oil production operations, discharge of coastal refinery effluent and spillage from oil tankers. Crude oil also enters the oceans by natural seepage from undersea locations. Particular attention has focused on major oil tanker spillages, notably involving the Torrey Canyon in 1969, the Amoco Cadiz in 1978 and the Exxon Valdez in 1990. As a result, the processes determining the fate of oil in seawater are reasonably well understood and have been reviewed by Atlas and Bartha. Initially, the oil spreads out as a film on the sea surface as a result of wind and wave action. The more volatile, lower molecular weight hydrocarbons are lost by evaporation. Polar compounds and the mono-aromatic hydrocarbons have an appreciable water solubility and are taken into solution. A key ancillary process is that of emulsification, since crude oil has a natural tendency to form emulsions in sea water. Such emulsions are usually of the oil-in-water type, but may also be of the water-in-oil type. The latter are often of the intractable 'chocolate mousse' type. Significant amounts of crude oil, particularly the higher molecular weight compounds. sink naturally, rolling along the ocean bottom picking up sand and shells and forming tarry balls which are resistant to degradation by any method. Hydrocarbons may also reach the bottom sediments by sorption onto suspended particles which ultimately settle on the sea floor. Spilt oil also undergoes chemical changes, particularly oxidation by free radical mechanisms initiated by sunlight.

The initial products of such reactions are hydroperoxides, and these in turn form compounds such as alcohols, acids and aldehydes, many of which have an appreciable water solubility. Polymerization also occurs to yield intractable tarry materials.

The bulk of spilt crude oil is biodegraded by the micro-organisms present in sea water. Emulsification to form oil-in-water emulsions yields small particles of crude oil that are biodegraded by bacteria, yeasts, fungi and actinomycetes. Many factors influence the rate of biodegradation, in particular temperature, dissolved oxygen concentration and the availability of nitrogen and phosphorus nutrients. Adapted micro-organisms are often found in ocean areas where crude oil spills are common. Zobell has calculated that where an adapted microbial population is available in well-aerated sea water at 20 to 30 °C, the rate of crude oil oxidation ranges from 0.02 to 0.2 g of oil oxidized/m²/day. The same author found experimentally that complete oxidation of 1.0 mg of hydrocarbon requires between 3 and 4 g of oxygen, i.e. it has a BOD of 3 to 4 mg oxygen/mg. Since the oxygen content of sea water is between 6 and 11 mg/liter, depending on salinity and temperature, this means that about 320 000 liters of sea water is required to oxidize one liter of crude oil. Crude oil

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contains hydrocarbons of well-defined generic types that are biodegraded at different rates. n-Alkanes are readily degraded in sea water, since many micro-organisms can utilize them. Branched-chain or iso-alkanes are less readily biodegraded but they do ultimately biodegrade. The degradation of cycloalkanes has not been extensively studied, but the ring structure is resistant to biodegradation. Aromatic hydrocarbons are also resistant to biodegradation, but a few micro-organisms are able to utilize them. High molecular weight compounds, the tars and asphaltenes, degrade very slowly.

(4) (12) (16) (17) (80) (81)

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4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : semistatic

Species: Oncorhynchus mykiss (Fish, fresh water)

Exposure period : 96 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : yes

Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"

Year : 2001 **GLP** : yes

Test substance : CAS 8002-05-9 Crude oil - 0.5% paraffinic

Method : LL 50 at 96 hr calculated using the Trimmed Spearman-Karber Method

(Hamilton, et al, 1977)

Result: Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5 and 10,

respectively for control, 1.4, 3.2, 8.5, 21 and 50 mg/l treatments. 96-hr LL $_{50}$ = 21 mg/l, 95% C.I: 16-28 mg/l (as nominal loading rate) 96-hour No Observed Effect Loading (NOEL) was 8.5 mg/l (observed.) Results are quoted in terms of 50% Lethal Loading (LL $_{50}$), the loading rate of test substance resulting in 50% mortality of the test species exposed to

the WAF.

Measured BTEX (mg/l) in test treatment (mg/l load)

Day	Control	1.4	3.2	8.5	21	<u>50</u>
0(new)	ND	0.129	0.382	0.835	1.94	4.73
1 (old)	ND	0.086	0.243	0.665	1.60	4.04
3 (new)	0.005	0.148	0.301	0.973	1.86	NA
4 (old)	0.005	0.127	0.255	0.813	1.70	NA

ND=not detected, NA=not analyzed due to 100% mortality

The Practical Quantitation Limit (PQL) was approximately 0.0035 mg/l (3.5ng/ml) which corresponds to the concentration of the lowest standard

analyzed.

Test condition: Test solutions were prepared as water accommodated fractions (WAF).

The control and dilution water was a reconstituted moderately hard water aerated prior to use. Water quality analyses for the dilution water was as

follows:

alkalinity = 72 mg/l as CaCO₃ hardness = 96 mg/l (as CaCO₃) specific conductance = 320 µmhos

pH = 7.7

dissolved oxygen = 8.6 ppm.

Based on results of range-finding tests for a similar petroleum product, measured loading rates of 1.4, 3.2, 8.5, 21 and 50 mg/l of crude oil to water were used to prepare test solutions for definitive toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 12-liter stoppered containers with approximately 20% vortex and less than 10% headspace volume. The WAFs were allowed to settle and cool to test temperature for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish were 85 days old at test initiation and were obtained from Pierce Associates Inc., West Buxton, ME, Lot 454. Loading of fish body mass to treatment was 0.551 g fish per liter of aqueous solution, mean length at termination was 4.1 cm (sd=0.2), and mean weight was 0.496 g (sd=0.078). Test vessels were 4-liter glass aspirator bottles with Teflon® covered neoprene

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stoppers. Two replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing at least 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEX. Mean water temperature range was 13.8 (sd=0.1) °C. Test photoperiod was 16 hrs. light and 8 hr dark, dissolved oxygen measurements ranged from 6.5 to 8.8 ppm, pH values between 6.8 and 7.8.

Reliability : (1) valid without restriction

(22)

Type : semistatic

Species: Oncorhynchus mykiss (Fish, fresh water)

Exposure period : 96 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : yes

Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"

Year : 2001 **GLP** : yes

Test condition

Test substance : CAS 8002-05-9 Crude oil 3% paraffinic

Method : LL 50 at 96 hr calculated using the Trimmed Spearman-Karber Method

(Hamilton, et al, 1977)

Result : Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5 and 10,

respectively for control, 2.7, 6.8, 16, 40 and 109 mg/l treatments. 96-hr LL $_{50}$ = 41 mg/l, 95% C.I: 30-55 mg/l (as nominal loading rate) 96-hour No Observed Effect Loading (NOEL) was 16 mg/l (observed.) Results are quoted in terms of 50% Lethal Loading (LL $_{50}$), the loading rate of test substance resulting in 50% mortality of the test species exposed to

the WAF.

Measured BTEX (mg/l) in test treatment (mg/l load)

Day	Control	2.7	6.8	16 Š	40	109
0 (new)	ND	0.093	0.366	0.541	1.36	2.02
1 (old)	0.050	0.066	0.184	0.411	0.857	1.84
3 (new)	ND	0.096	0.272	0.585	1.28	2.01
4 (old)	0.031	0.084	0.221	0.484	1.03	NA

ND=not detected, NA=not analyzed due to 100% mortality

Guideline/protocol deviations: DO level was 53%, not 60% of saturation.

aerated prior to use. Water quality analyses for the dilution water was as

Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a reconstituted moderately hard water

follows:

alkalinity = 78 mg/l as CaCO₃ hardness = 105 mg/l (as CaCO₃) specific conductance = 324 µmhos

pH = 7.6

dissolved oxygen = 8.4 ppm.

Nominal loading rates of 0, 3.8, 16 and 74 mg/l were used to prepare test

solutions for range-finding toxicity tests.

Based on results of range-finding tests, measured loading rates of 0, 2.7, 6.8, 16, 40 and 109 mg/l of crude oil to water were used to prepare test solutions for definitive toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 13-

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liter stoppered containers with approximately 20% vortex and less than 10% headspace volume. The WAFs were allowed to settle and cool to test temperature for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish were 85 days old at test initiation and were obtained from Pierce Associates Inc., West Buxton, ME, Lot 454. Loading of fish body mass to treatment was 0.4 g fish per liter of aqueous solution, mean length at termination was 3.9 cm (sd=0.2), and mean weight was 0.466 g (sd=0.034). Test vessels were 4-liter glass aspirator bottles with Teflon® covered neoprene stoppers. Two replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing at least 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEX. Water temperature range was 14.3 to 14.9 °C. Test photoperiod was 16 hrs. light and 8 hr dark, dissolved oxygen measurements ranged from 5.4 to 8.8 ppm, pH values between 6.8 and 7.8.

Reliability : (1) valid without restriction

(27)

Type : static

Species: Cyprinodon variegatus (Fish, estuary, marine)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 2900 - 80000 measured/nominal

Analytical monitoring : yes

Method: Procedure as detailed in paper by Anderson (see Reference)

Year : 1974 GLP : no data

Test substance: South Louisiana and Kuwait crude oils

Method: The test species was the Sheepshead Minnow. Dispersions of two crude

oil samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5

concentrations. Figures quoted are for the loading rates.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data

are ranked by API crude oil task group as '3', not reliable.

Result : The results were as follows:

For Kuwait crude:

48-hour $LC_{50} = 80\ 000+\ mg/l$ 96-hour $LC_{50} = 80\ 000+\ mg/l$ For South Louisiana crude: 48-hour $LC_{50} = 33\ 000\ mg/l$ 96-hour $LC_{50} = 29\ 000\ mg/l$

Parallel tests run with water-soluble fractions (WSF) failed to produce any

meaningful results.

Reliability : (3) invalid

(2)

Date: November 15, .2003

Type : static

Species: Fundulus similis (Fish, estuary, marine)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 6000 - 14800 measured/nominal

Method : Procedure as detailed in paper by Anderson (see Reference)

Year : 1974 **GLP** : yes

Test substance : South Louisiana and Kuwait crude oils

Method: The test species was the Longnose Killifish. Dispersions of two crude oil

samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5

concentrations. Figures quoted are for the loading rates.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data

are ranked by API crude oil task group as '3', not reliable.

Result : The results were as follows:

For Kuwait crude:

48-hour $LC_{50} = 14\ 800\ mg/l$ 96-hour $LC_{50} = 14\ 800\ mg/l$ For South Louisiana crude: 48-hour $LC_{50} = 6000\ mg/l$ 96-hour $LC_{50} = 6000\ mg/l$

Reliability : (3) invalid

(2)

Type : static

Species: Menidia beryllina (Fish, estuary, marine)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 3700 - 9400 measured/nominal

Analytical monitoring : no

Method: Procedure as detailed in paper by Anderson (see Reference)

Year : 1974 **GLP** : yes

Test substance : South Louisiana and Kuwait crude oils

Method : The test species was the Tidewater Silverside. Dispersions of two crude oil

samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5

concentrations. Figures quoted are for the loading rates.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These

data are ranked by API crude oil task group as '3', not reliable.

Result: The results were as follows:

For Kuwait crude:

48-hour $LC_{50} = 15\ 000\ mg/l$, 96-hour $LC_{50} = 9400\ mg/l$

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For South Louisiana crude:

48-hour $LC_{50} = 5000 \text{ mg/l}$ 96-hour $LC_{50} = 3700 \text{ mg/l}$

Source : CONCAWE Bruxelles

Reliability : (3) invalid

(2)

Type : static

Species: Salmo gairdneri (Fish, estuary, fresh water)

Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes

Method : Procedure as detailed in paper by Lockhart, Danell and Murray (see

Reference)

Year : 1987 **GLP** : yes

Test substance : Norman Wells crude oil

Method : The test species was the Rainbow Trout. A water-soluble fraction (WSF) of

the test substance was prepared by adding crude oil to water at a concentration of 12.5 ml/l and stirring for 2 hours. After settling for 72 hours, groups of 5 fish were exposed to solutions containing 20, 30, 40 and

50% WSF. Three series of tests were run:

(a) with closed vessels(b) with open vessels, and(c) with aerated vessels.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '2', reliable with

restrictions.

Result : The results were as follows:

48-hour LC_{50} (open vessel) = 10.4 mg/l 48-hour LC_{50} (closed vessel) = 11.6 mg/l

based on measurements of dissolved hydrocarbons made at the beginning and end of the 48-hour period. No fish died in the aerated vessels, and hydrocarbons were undetectable in solution at the 48-hour

time point in these studies.

Source : CONCAWE Bruxelles
Reliability : (2) valid with restrictions

(44)

Type : static

Species: Salmo gairdneri (Fish, estuary, fresh water)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 291 measured/nominal

Analytical monitoring : no

Method: procedure as detailed in paper by Westlake (see Reference)

Year : 1991 GLP : yes

Test substance: Forties crude oil

Method: The test species was the Rainbow Trout. Dispersions of oil in water at 8

concentrations in the range 57 to 1330 mg/l were prepared by shaking the constituents together in closed jars for 5 minutes using a reciprocating

table. Fish tests were run in open vessels.

Date: November 15, .2003

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '3', not reliable.

Result: The results were as follows:

24-hour $LC_{50} = 756$ mg/l 96-hour $LC_{50} = 291$ mg/l 168-hour $LC_{50} = 133$ mg/l

As the tests were run in open vessels, significant evaporative losses of lower hydrocarbons will have occurred and hence the measured LC_{50}

values underestimate the true ecotoxicity.

Source : CONCAWE Bruxelles

Reliability : (3) invalid

(78)

Type : static

Species: Salmo gairdneri (Fish, estuary, fresh water)

Unit : mg/l

LC50 : 258 measured/nominal

Analytical monitoring : no

Method : procedure as detailed in paper by Westlake (see Reference)

GLP : yes

Test substance: Forties crude oil

Method: The test species was the Rainbow Trout.

Crude oil was added to water at 8 concentrations in the range 57 to 1330 mg/l in open vessels fitted with shielded propeller stirrers. A constant rate of stirring was maintained during the 7-day exposure period to the fish.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data

are ranked by API crude oil task group as '3', not reliable.

Result: The results were as follows:

24-hour $LC_{50} = 557$ mg/l 96-hour $LC_{50} = 258$ mg/l 168-hour $LC_{50} = 253$ mg/l

As the tests were run in open vessels, significant evaporative losses of lower hydrocarbons will have occurred and hence the measured LC_{50}

values underestimate the true ecotoxicity.

Source : CONCAWE Bruxelles

Reliability : (3) invalid

(78)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Species : Crangon crangon (Crustacea)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 27 - 110 measured/nominal

Date: November 15, .2003

Analytical monitoring : no

Method: Procedure as detailed in paper by Franklin and Lloyd (see Reference)

Year : 1982 GLP : no data

Test substance: Samples of 8 crude oils

Method : The test species was the Brown Shrimp. Groups of 20 shrimps were

exposed to nominal concentrations in the range 17 to 3400 mg/l of each crude oil in sea water. After addition of the crude oil, the solutions were stirred at a constant rate using a shielded stirrer. Crude oil solutions were

renewed after 48 hours. Mortalities were recorded daily.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since no analytical determinations were reported, these data are ranked by

API crude oil task group as '2', reliable with restrictions.

Result: The lowest LC₅₀ value was obtained for Brent crude and the highest for

Thistle crude. Other North Sea crude oils tested were from the Argyll, Auk, Beryl, Claymore, Ekofisk, Forties, Montrose, Murchison and Piper fields.

Source : CONCAWE Bruxelles
Reliability : (2) valid with restrictions

(32)

Species : Crangon crangon (Crustacea)

Exposure period : 96 hour(s)
Unit : mg/l

LC50 : 41 - 119 measured/nominal

Analytical monitoring : no

Method : Procedure as detailed in paper by Franklin and Lloyd (see Reference)

Year : 1982 GLP : no data

Test substance : Samples of 8 crude oils

Method: The test species was the Brown Shrimp. Groups of 20 shrimps were

exposed to nominal concentrations in the range 17 to 3400 mg/l of each crude oil in sea water. After addition of the crude oil, the solutions were stirred at a constant rate using a shielded stirrer. Crude oil solutions were

renewed after 48 hours. Mortalities were recorded daily.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since no analytical determinations were reported, these data are ranked by

API crude oil task group as '2', reliable with restrictions.

Result: The lowest LC₅₀ value was obtained for Abu Dhabi crude and the highest

for Iranian Light crude. Other crude oils tested were from Libya, Saudi

Arabia, Nigeria, Kuwait, Iraq and Iran (heavy crude).

Source : CONCAWE Bruxelles

Reliability : (2) valid with restrictions

(33)

Species : Daphnia magna (Crustacea)

Exposure period : 24 hour(s)
Unit : mg/l

EC50 : 36 measured/nominal

Analytical monitoring : no

Method : OECD Guide-line 202

Year : 1981

Date: November 15, .2003

GLP : yes

Test substance: Forties crude oil

Method : Dispersions of oil in water at 5 concentrations in the range 10 to 100 mg/l

were prepared by shaking the constituents together in closed jars for 5 minutes using a reciprocating table. Groups of 10 daphnids were exposed

to the resulting solutions. Tests were run in open vessels.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC $_{50}$ values are expected to be lower than those cited. These data are ranked by API crude oil task group as '3', not reliable.

Result: The results were as follows: 24-hour $EC_{50} = 36$ mg/l. As the tests were

run in open vessels, significant evaporative losses of lower hydrocarbons will have occurred and hence the measured EC_{50} value underestimates the

true ecotoxicity.

Source : CONCAWE Bruxelles

Reliability : (3) invalid

(78)

Species : Daphnia magna (Crustacea)

Exposure period : 24 hour(s)
Unit : mg/l

EC50 : = 42 measured/nominal

Analytical monitoring : no

Method : OECD Guide-line 202

Year : 1981 **GLP** : yes

Test substance: Forties crude oil

Method : Crude oil was added to water at 8 concentrations in the range 10 to 100

mg/l in open vessels fitted with shielded propeller stirrers. A constant rate of stirring was maintained during the 24-hour exposure period to groups of

10 daphnids.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC_{50} values are expected to be lower than those cited. These data are ranked by API crude oil task group as '3', not reliable.

Result: The results were as follows: 24-hour $EC_{50} = 42 \text{ mg/l}$. As the tests were

run in open vessels, significant evaporative losses of lower hydrocarbons will have occurred and hence the measured EC_{50} value underestimates the

true ecotoxicity.

Source : CONCAWE Bruxelles

Reliability : (3) invalid

(78)

Туре

Species : Mysidopsis bahia (Crustacea)

Exposure period : 96 hour(s)
Unit : mg/l

LL50 : 618 measured/nominal

Analytical monitoring : yes

Method : 40 CFR Part 797.1930, Mysid Shrimp Acute Test

Year : 1993

Date: November 15, .2003

GLP : yes

Test substance: Kuwait crude oil

Method: Tests were carried out in polypropylene containers using water

accommodated fractions prepared by physical dispersion. These were renewed daily and kept tightly covered with teflon sheets. Loading rates of 340, 695, 1550, 3470 and 7740 mg/l were used in this study. The test

species was the estuarine mysid, Mysodopsis bahia.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without

restrictions.

Result: A lethal loading rate concentration LL₅₀ (96h) of 618 mg/l was calculated

from data presented in the original publication after consultation with the main author. Weight percent distribution for the crude oil components with BP up to 425 °F are: paraffins: 68.56%; aromatics: 15.69 and naphthenes:

11.86%.

Source : CONCAWE Bruxelles Reliability : (1) valid without restriction

Species : Holmesiysis costata

Exposure period : 96 hour(s)
Unit : mg/l

LL50 : 39.5 measured/nominal

Analytical monitoring : yes

Method : 40 CFR Part 797.1930, Mysid Shrimp Acute Test

Year : 1993 **GLP** : yes

Test substance: Kuwait crude oil

Method: Tests were carried out in polypropylene containers using water

accommodated fractions prepared by physical dispersion. These were renewed daily, and kept tightly covered with teflon sheets. Loading rates of

11.6, 34.3, 99.1, 284 and 862 mg/l were used in this study. The test

species was the kelp forest mysid, Holmesimysis costata.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without

restrictions

Result : A lethal loading rate concentration, LL₅₀ (96h) of 39.5 mg/l was calculated

from data presented in the original publication after consultation with the main author. Weight percent distribution for the crude oil components with BP up to 425 °F are: paraffins: 68.56%; aromatics: 15.69 and naphthenes:

11.86%.

Source : CONCAWE Bruxelles
Reliability : (1) valid without restriction

(6)

Type : semistatic

Species : Kelp Forest Mysid (Holmesymis costata)

Exposure period : 96 hour(s)
Unit : mg/l
Limit Test : no
Analytical monitoring : yes

Date: November 15, .2003

Method : USEPA 600 4-90-027

Year : 2002 GLP : yes

Test substance : CAS 8002-05-9 Crude oil, 0.5% paraffinic

Method : LL 50 at 96 hr calculated maximum likelihood analysis based on D.J.

Finney, 1971

Result: Mortality (no. of deaths/treatment) at 96 hrs: 0, 2, 2, 1, 15 and 20,

respectively for control, 0.14, 0.28, 1.4, 3.5, and 11 mg/l treatments. 96-hr LL $_{50}$ = 2.7 mg/l, 95% C.I: 2.2-3.6 mg/l (as actual loading rate) Results are quoted in terms of 50% Lethal Loading (LL $_{50}$), the loading rate of test substance resulting in 50% mortality of the test species exposed to

the WAF.

Measured BTEX (mg/l) in test treatment (mg/l load)

Day	Control	0.14	0.28	1.4	3.5	<u>11</u>
0 (new)	ND	ND	ND	0.049	0.167	0.500
1 (old)	ND	ND	ND	0.0118	0.053	0.239
3 (new)	0.047	ND	0.003	0.0586	0.203	NA
4 (old)	ND	ND	ND	0.017	0.0939	NA

ND=not detected, NA=not analyzed due to 100% mortality The practical quantitation limit was 0.003 mg/l. Measured soluble concentrations of BTEX may have been reduced by the presence of brine shrimp in the test chambers

Test condition

Test species: Juvenile Mysids used in the test were 1day old and were obtained from A.K. Siewers, Santa Cruz, CA, USA; the Mysids were acclimated for 3 days under laboratory conditions in seawater supplied by the organism vendor and fed from in-house cultures of Artemia salina nauplii that were less than 24 hours old. Test organisms were fed brine shrimp once per day during the study.

Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. New test solutions were prepared for each 24-hour interval throughout the duration of the test. The control and dilution water was natural seawater collected from Manasquan Inlet, NJ, (NJDEP designated collection site) with a salinity of 33% and passed through 0.45 um filters prior to use.

Based on results of range-finding tests for a similar petroleum product, actual loading rates of 0, 0.14, 0.28, 1.4, 3.5 and 11 mg/l of crude oil to water were used to prepare test solutions for definitive toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in appropriate-sized, stoppered containers with approximately 20% vortex and less than 10% headspace volume. The WAFs were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. A new WAF was prepared 24 hours prior to each renewal. Water quality (temperature, pH, salinity, and dissolved oxygen) measurements were recorded daily on the new solutions. Temperature, pH, and dissolved oxygen measurements were recorded on the old solutions (composite of replicates).

Test conditions: Two replicate chambers per treatment were tested. Each replicate contained ten mysids. Replicate chambers were 500 ml glass jars with Teflon screw lids, containing approximately 450 ml of test solution with no headspace. All test and control solutions were replenished daily. Renewals were accomplished by transferring the mysids to fresh solution in a second set of test chambers on days 1, 2 and 3.

Id: 8002-05-9

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Light: 16-hour light per day and a daylight intensity that ranged from 494 to 720 Lux.

Test temperature: 11.9°C (S.D.=0.2).

Water chemistry:

Dissolved oxygen ranged from 6.4 - 7.0 mg/l

pH ranged from 8.1 - 8.5 salinity ranged from 32 - 34 ppt.

Freshly prepared and old WAF test solutions were analyzed by GC-FID for

concentrations of BTEX.

Reliability : (1) valid without restriction

3 (26)

Type : semistatic

Species : Kelp Forest Mysid (Holmesymis costata)

Exposure period : 96 hour(s)
Unit : mg/l
Limit Test : no
Analytical monitoring : yes

Method : USEPA 600 4-90-027

Year : 2001 GLP : yes

Test substance : CAS 8002-05-9 Crude oil, 3% paraffinic

Method : LL ₅₀ at 96 hr calculated maximum likelihood analysis based on D.J.

Finney, 1971

Result: Mortality (no. of deaths/treatment) at 96 hrs: 1, 2, 3, 6, 20 and 20,

respectively for control, 0.6, 1.7, 3.6, 8.3 and 21 mg/l treatments. 96-hr $LL_{50} = 4.1\,$ mg/l, 95% C.I: 3.3-5.3 mg/l (as actual loading rate) Results are quoted in terms of 50% Lethal Loading (LL_{50}), the loading rate of test substance resulting in 50% mortality of the test species exposed to

the WAF.

Measured BTEX (mg/l) in test treatment (mg/l load)

Day	Control	0.6	1.7	3.6	8.3	<u>21</u>
0 (new)	ND	ND	ND	ND	0.072	0.247
1 (old)	ND	ND	ND	ND	ND	0.057
3 (new)	ND	ND	ND	0.019	NA	NA
4 (old)	ND	ND	ND	0.023	NA	NA

ND=not detected, NA=not analyzed due to 100% mortality The practical quantitation limit was 0.003 mg/l. Measured soluble

concentrations of BTEX may have been reduced by the presence of brine

shrimp in the test chambers

Test condition: Test species: Juvenile Mysids used in the test were 1day old and were

obtained from A.K. Siewers, Santa Cruz, CA, USA; the Mysids were acclimated for 3 days under laboratory conditions in seawater supplied by the organism vendor and fed from in-house cultures of Artemia salina nauplii that were less than 24 hours old. Test organisms were fed brine

shrimp once per day during the study.

Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. New test solutions were prepared for each 24-hour interval throughout the duration of the test. The control and dilution water was natural seawater collected from Manasquan Inlet, NJ,

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Date: November 15, .2003

(NJDEP designated collection site) with a salinity of 33% and passed through 0.45 um filters prior to use.

Nominal loading rates of 0, 1.8, 19 and 105 mg/l were used to prepare test solutions for range-finding toxicity tests.

Based on results of range-finding tests, actual loading rates of 0, 0.6, 1.7, 3.6, 8.3 and 21 mg/l of crude oil to water were used to prepare test solutions for definitive toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in appropriate-sized, stoppered containers with approximately 20% vortex and less than 10% headspace volume. The WAFs were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. A new WAF was prepared 24 hours prior to each renewal. Water quality (temperature, pH, salinity, and dissolved oxygen) measurements were recorded daily on the new solutions. Temperature, pH, and dissolved oxygen measurements were recorded on the old solutions (composite of replicates).

Test conditions: Two replicate chambers per treatment were tested. Each replicate contained ten mysids. Replicate chambers were 500 ml glass jars with Teflon® screw lids, containing approximately 450 ml of test solution with no headspace. All test and control solutions were replenished daily. Renewals were accomplished by transferring the mysids to fresh solution in a second set of test chambers on days 1, 2 and 3.

Light: 16-hour light per day and a daylight intensity that ranged from 494 to 720 Lux.

Test temperature: 12.3°C (S.D.=0.1).

Water chemistry:

Dissolved oxygen ranged from 6.4 - 7.0 mg/l

pH ranged from 8.1 - 8.5 salinity ranged from 32 – 33 ppt.

Freshly prepared and old WAF test solutions were analyzed by GC-FID for

concentrations of BTEX.

Reliability : (1) valid without restriction

(25)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Anabaena doloilum (Algae)

Endpoint : growth rate
Exposure period : 15 day(s)
Unit : mg/l
Analytical monitoring : yes

Method: Procedure as detailed by Gaur and Singh (see Reference)

Year : 1989 GLP : no data

Test substance : Assam crude oil

Method : Tests were run using:

(a) a water-soluble fraction (WSF) of the test substance, and

(b) the test substance in equilibrium with the algal suspension medium. To prepare the WSF, crude oil was added to the sterilized medium in the ratio 1:20, and was stirred in closed bottles for 12 hours. After a 4-hour separation period the aqueous phase was separated and diluted for the tests. In the direct loading tests, crude oil was applied to absorbent pads and these were held in the culture suspension. Analysis was done by gas

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chromatography, following solvent extraction of the aqueous phase using

n-pentane.

Remark: These data are included to provide supporting evidence of the expected

cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without

restrictions.

Result: The WSF method gave a mean 15-day EC₅₀ of 9.06 mg/l, and the direct

loading (or whole oil) method gave a 15-day EC_{50} of 5.73 mg/l, both based on measured dissolved hydrocarbon concentrations in the aqueous phase.

Source : CONCAWE Bruxelles
Reliability : (1) valid without restriction

(34)

Species: Macrocystis pyrifera

Exposure period : 48 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : yes
Year : 2001
GLP : yes

Test substance : CAS 8002-05-9 Crude oil - 0.5% paraffinic

Method : The method used was described by Chapman, G.A., D.L. Denton and J.M.

Lazorchak. (1995).

Statistical method

Lethal Loading and Effect Loading: Norberg-King, T.J., A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). July 1993. U.S. Environmental Protection

Agency, Environmental Research Laboratory, Duluth MN.

Result: 48 hour results, based on mg of test substance per liter of natural seawater

Germination LL ₅₀ (mg/l)	Tube Length EL ₅₀ (mg/l)	NOEL	nation LOEL (mg/l)	NOEL	LŎEL
122	122	52	146	18	52

Analytical results

Nominal (mg/l)	Measured Concentration (mg/l as BTEX)		
	Day 0	Day 2	
Control	none detected	none detected	
18	0.542	0.478	
52	1.82	1.23	
146	5.91	3.27	
415	15.2	5.88	
1185	20.0	11.3	

Practical Quantitation Limit (PQL) was approximately 0.0035 mg/l

(3.5 ng/ml)

Test condition: Individual test treatment solutions were prepared as Water Accommodated

Fractions (WAFs). Actual loading levels of test substance in natural seawater (dilution water) were 18 mg/l, 52 mg/l, 146 mg/l, 415 mg/l and 1185 mg/l. A control treatment consisting of natural seawater with no test

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substance was also prepared. The WAFs were prepared by adding the appropriate amount of test substance to natural seawater in glass aspirator bottles and stirring on magnetic stirplates for 24 hours. Natural seawater was filtered through a 0.45 µm filter, then a 0.2 µm filter (collected from Manasquan Inlet, NJ, NJDEP designated collection site).

The salinity of the seawater was 34 ± 2 ppt.

Ammonia $(NH_3 + NH_4+) = 0 \text{ mg/l}$

Nitrite $(NO_2-) = <0.3 \text{ mg/l}$

Nitrate (N) = 1 mg/l.

The mixtures were allowed to settle and cool to test temperature for 1.25 hour before removing the aqueous portions (WAFs) for testing. Samples of the WAFs prepared at each loading level were taken on Day 0 and at termination from each treatment (composite of replicates). The samples were analyzed for benzene, toluene, ethylbenzene and o-xylene (BTEX), all hydrocarbons detected in the entire chromatographic run were quantified versus the BTEX standards and the reported results reflect the sum of all quantified compounds. Samples were taken with no headspace in volatile organic analysis (VOA) vials.

Test temperature was 14.0 (sd=0.3) °C.

The kelp sporophylls (reproductive blades) were received from A. K. Siewers, Santa Cruz, CA 95060, packed on ice at 7.5°C, the day after they were harvested. The sporophylls were rinsed with 0.2 µm filtered seawater at 14°C and blotted dry with paper towels. They were then desiccated at 14°C for one hour. The sporophylls were rinsed again with 0.2µm filtered seawater at 14°C. Approximately 20 of the sporophylls were placed into a one liter glass beaker containing 800 ml of 0.2 µm filtered seawater at 14°C. The blades were left in the beaker for 30 minutes while the zoospores released. The blades were removed and the spore solution was allowed to settle for 20 minutes. 250 ml was poured from the top of the beaker, this was the spore stock. The zoospores were released from the sporophylls and inoculated into test chambers within a two hour period. The zoospore release was initiated within 24 hours of sporophyll harvest.

Five replicate chambers for each dose treatment consisted of 500 ml size glass jars sealed with Teflon® lined screw type lids, containing approximately 450 ml of test solution to minimize headspace and prevent loss of volatile components.

A pre-cleaned 2.5 cm x 7.6 cm glass slide was placed in the bottom of each jar prior to adding test solution. The initial concentration was approximately 7.5 x 10³ zoospores/ml in each replicate chamber. The spore stock density was determined as follows: 1 ml of the stock and 1 ml of glacial acetic acid were added to 8 ml of 0.2 µm filtered seawater in a graduated cylinder. The solution was mixed and counts were performed using a hemacytometer at 100x. Each test chamber, containing approximately 7500 spores, was labeled to show the study number, loading level, replicate and randomization number. Each test and control chamber were observed for germination of the gametophyte spores and length of the embryonic gametophyte germination tube after approximately 48 hrs. The following procedure was used. The glass slide was removed from the jar and the bottom of the slide was dried with a paper towel. A 24 x 50 mm cover slideslip was placed onto the slide and the edges were dabbed. Germination was considered successful if a germ tube was present on the settled zoospore. Germination was considered unsuccessful if no germ tube was visible.

400x magnification was used to differentiate germination and measure the tubes. A spore had to have a protuberance that extended at least one

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spore diameter from the edge of the spore to be considered germinated. The first 100 spores, which were easily identifiable, were counted while moving the slide across the field of view of the microscope. Measurements were made by moving the slide and choosing the first 10 spores with straight germ tubes within the same focal plane of the microscope that were closest to the scale in the ocular micrometer. The ocular micrometer was calibrated with a stage micrometer at 400x to assure accurate measurements. The micrometer was calibrated from 3 to 25 μm . The total length of the tube from the edge of the original spore membrane was measured.

Water quality (temperature, pH, dissolved oxygen, and salinity) measurements were made on each treatment on Day 0 and at termination (composite of replicates). BTEX total concentration at termination was at least 80% of the initial concentration.

The study was deemed acceptable. Mean control germination was > 70% in the controls. Mean germination tube length was > 10 μ m in the controls.

Reliability : (1) valid without restriction

(10)(24)

Species : Marine algae (Macrocystit pyrifera)

Exposure period : 48 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : yes

Method : Chapman, G.A., D.L. Denton and J.M. Lazorchak. 1995

Year : 2001 **GLP** : yes

Test substance : CAS 8002-05-9 Crude oil 3% paraffinic

Method : Lethal Loading and Effect Loading: Norberg-King, T.J., A Linear

Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). July 1993. U.S. Environmental Protection

Agency, Environmental Research Laboratory, Duluth MN.

Result: 48 hour results, based on mg of test substance per liter of natural seawater

(95%CI)

	Tube Length EL ₅₀				_
528 (497-558)	311 (257-403)	53	130	23	53

Analytical results

Nominal (mg/l)	ntration (mg/l as BTEX)	
	Day 0	Day 2
Control	none detected	none detected
23	0.047	none detected
53	0.586	0.039
130	1.09	0.428
323	3.35	1.92
802	5.03	4.43

Practical Quantitation Limit (PQL) was approximately 0.0035 mg/l (3.5ng/ml)

Spore concentration was 1000 spore/ml, instead of 7500 spore/ml, as

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Test condition

required. However total spore number per replicate was adequate sample size for determination of end points.

Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Actual loading levels of test substance in natural seawater (dilution water) were 23 mg/l, 53 mg/l, 130 mg/l, 323 mg/l and 802 mg/l. A control treatment consisting of natural seawater with no test substance was also prepared. The WAFs were prepared by adding the appropriate amount of test substance to natural seawater in glass aspirator bottles and stirring on magnetic stirplates for 24 hours. Natural seawater was filtered through a 0.45 µm filter, then a 0.2 µm filter (collected from Manasquan Inlet, NJ, NJDEP designated collection site). The salinity of the seawater was 34 ± 2 ppt. The mixtures were allowed to settle and cool to test temperature for 1 hour before removing the aqueous portions (WAFs) for testing. Samples of the WAFs prepared at each loading level were taken on Day 0 and at termination from each treatment (composite of replicates). The samples were analyzed for benzene, toluene, ethylbenzene and o-xylene (BTEX), all hydrocarbons detected in the entire chromatographic run were quantified versus the BTEX standards and the reported results reflect the sum of all quantified compounds. Samples were taken with no headspace in volatile organic analysis (VOA) vials. Mean test temperature was 14.1 (sd = 0.3) °C.

The kelp sporophylls (reproductive blades) were received from A. K. Siewers, Santa Cruz, CA 95060, packed on ice at 12.2°C, the day after they were harvested. The sporophylls were rinsed with 0.2 µm filtered seawater at 14°C and blotted dry with paper towels. They were then desiccated at 14°C for one hour. The sporophylls were rinsed again with 0.2µm filtered seawater at 14°C. Approximately 20 of the sporophylls were placed into a one liter glass beaker containing 800 ml of 0.2 µm filtered seawater at 14°C. The blades were left in the beaker for 30 minutes while the zoospores released. The blades were removed and the spore solution was allowed to settle for 25 minutes. 250 ml was poured from the top of the beaker, this was the spore stock. The zoospores were released from the sporophylls and inoculated into test chambers within a two hour period. The zoospore release was initiated within 24 hours of sporophyll harvest. Five replicate chambers for each dose treatment consisted of 500 ml size glass jars sealed with Teflon® lined screw type lids, containing approximately 450 ml of test solution. A pre-cleaned 2.5 cm x 7.6 cm glass slide was placed in the bottom of each jar prior to adding test solution. The initial concentration was approximately 1.0 x 10³ zoospores/ml in each replicate chamber. The spore stock density was determined as follows: 1 ml of the stock and 1 ml of glacial acetic acid were added to 8 ml of 0.2 µm filtered seawater in a graduated cylinder. The solution was mixed and counts were performed using a hemacytometer at 100x. Each test chamber, containing approximately 1000 spores, was labeled to show the study number, loading level, replicate and randomization number. Each test and control chamber was observed for germination of the gametophyte spores and length of the embryonic gametophyte germination tube. The following procedure was used. The glass slide was removed from the jar, and the bottom of the slide was dried with a paper towel. A 24 x 50 mm cover slideslip was placed onto the slide and the edges were dabbed. Germination was considered successful if a germ tube was present on the settled zoospore. Germination was considered unsuccessful if no germ tube was visible. 400x magnification was used to differentiate germination and measure the tubes. A spore had to have a protuberance that extended at least one spore diameter from the edge of the spore to be considered germinated. The first 100 spores, which were easily identifiable, were

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counted while moving the slide across the field of view of the microscope. Measurements were made by moving the slide and choosing the first 10 spores with straight germ tubes within the same focal plane of the microscope that were closest to the scale in the ocular micrometer. The ocular micrometer was calibrated with a stage micrometer at 400x to assure accurate measurements. The micrometer was calibrated from 3 to 25 µm. The total length of the tube from the edge of the original spore membrane was measured. Water quality (temperature, pH, dissolved oxygen, and salinity) measurements were made on each treatment on Day 0 and at termination (composite of replicates). BTEX total concentration at termination was at least 80% of the initial concentration. The study was deemed acceptable. Mean control germination was > 70% in the controls. Mean germination tube length was > 10 um in the controls.

Reliability : (1) valid without restriction

(10)(23)

4.9 ADDITIONAL REMARKS

Source

Reliability

Memo: Effect of crude and refined oil on fresh and sea water organisms

Remark: Burks has extensively reviewed the effects of crude and refined oils on

organisms found in fresh and sea water. He noted that where spillages occur the non-mobile species suffer the greatest mortality, whereas fish species can often escape from the affected region. The extent of the initial mortality depends on the chemical nature of the oil, the location, and the physical conditions, particularly the temperature and wind velocity. Most affected freshwater and marine communities recover from the effects of an oil spill within a year. The occurrence of biogenic hydrocarbons in the world's oceans is well recorded. They have the characteristic isoprenoid structure, and measurements made in water columns indicate a background concentration of 1.0 to 10 µl/l. The higher molecular weight

materials are dispersed as particles, with the highest concentrations of about 20 µl/l occurring in the top 3 mm layer of water.

A wide variation in the response of organisms to oil exposures has been noted. The larvae of fish and crustaceans appear to be most susceptible to the water-soluble fraction of crude oil. Exposures of plankton and algae have indicated that certain species of diatoms and green algae are inhibited, whereas microflagellates are not.

For the most part, molluscs and most intertidal worm species appear to be tolerant of oil contamination.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4', reliability not assigned

CONCAWE Bruxelles
(4) not assignable

(8)

Memo : Effect of crude oil on Lobster larvae

Remark : The effects of emulsified South Louisiana crude oil on the development of

American Lobster, Homarus americanus, was investigated during the development of the first four larval stages over a 15-day period. Hatched larvae were exposed to concentrations of 0, 0.1 and 1.0 ppm crude oil, six times per day for periods ranging from 0.8 to 5.6 minutes, using a flow-through system. The tests showed that 1.0 ppm crude oil was a sub-lethal

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concentration for the lobster larvae and that no significant effects were found at 0.1 ppm. At 0.1 ppm crude oil, the survival rate of the larvae was comparable with that of the controls, but at 1.0 ppm the survival value was about 50% that of the control larvae. Other effects observed at 1.0 crude oil included: (a) lethargy, reduced feeding and lack of the characteristic aggression of the control animals, (b) an increase in development time from 12 to 15 days, and (c) a change in the pigmentation of the larvae from the normal pale blue, almost transparent, color to a sharp red appearance.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4',

reliability not assigned CONCAWE Bruxelles

Source : CONCAWE Bruxelle Reliability : (4) not assignable

(31)

Memo : Effect of crude oil on Sea urchin eggs

Remark : Falk-Petersen has studied the effects of the water-soluble fraction (WSF) of

Ekofisk crude oil on the development of the eggs of two sea urchin species, Strongylocentrotus pallidus and S. droebachiensis. The WSF was prepared by shaking Ekofisk crude oil and sea water in the ratio 1:9 for 5 minutes, allowing to separate for 20 hours, and using the aqueous phase

for the studies.

Fertilized eggs were exposed to dilutions of the WSF for up to 9 days at 3 to 5 °C. Embryos and larvae were examined regularly by scanning and transmission electron microscopy after fixing in 2% OsO_4 in sea water. Concentrations of 30% WSF, corresponding to 13 ppm dissolved oil, did not impair either development or the ultrastructure of the stages from egg to pluteus. However, significant effects on development were found at both 40% WSF (17 ppm oil) and 50% WSF (21 ppm oil). At these levels, the larvae filled with degenerating cells, and differentiation of the intestine and skeletal growth were inhibited compared with the control larvae.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4',

reliability not assigned CONCAWE Bruxelles

Source : CONCAWE Bruxe Reliability : (4) not assignable

(28)

Memo : Effect of crude oil on eggs

Remark: Crude oil from 4 locations was applied externally at different loadings to

batches of 30 fertilized eggs of mallard ducks on day 3 of incubation at 37.5 °C. Eggs were candled each day to determine mortality, and dead embryos examined for abnormalities. On day 18 of incubation, surviving embryos were examined for external malformations. The observed LD50 values for crude oils from Kuwait, Prudhoe Bay, South Louisiana and Texas were 2.2, 8.3, 1.3 and 5.5 μ /egg, respectively. The South Louisiana and Texas crudes both produced significantly reduced growth, both above and below the LD₅₀ value; preformed embryos were also found with both these crudes.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4',

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reliability not assigned

Reliability : (4) not assignable

(37)

Memo : Poorly water soluble mixtures

Remark: For the assessment of the ecotoxicity of poorly water soluble mixtures of

hydrocarbons as found in petroleum products, it is now generally accepted that results should be expressed in terms of the "loading rate". The "loading rate" may be defined as the amount of the product which must be equilibrated with the aqueous test medium in order to produce a specified level of effect. Studies in which the results are expressed in terms of the measured concentrations of hydrocarbons in dilutions of "water soluble fractions (WSF)" do not allow the ecotoxicity of a product to be expressed in terms of the amount of that product required to produce a particular effect and, therefore, such results are not comparable to results

obtained with other substances.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1',

reliable without restrictions.

Source : CONCAWE Bruxelles
Reliability : (1) valid without restriction

(13) (35) (79)

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5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}

Value : > 5000 mg/kg bw

Species : rat

Strain : Sprague-Dawley Sex : male/female

Number of animals : 5

Vehicle : None, undiluted

Doses : Single dose: 5000 mg/kg

Year : 1984 GLP : no data

Test substance : Beryl crude (Light crude oil)

Method : Groups of five male and five female Sprague-Dawley rats were dosed once

by oral gavage with the test material at a dose level of 5 g/kg. The animals were observed frequently on the day of treatment and daily thereafter for 14 days. The animals were weighed on the day of treatment and again 7

and 14 days later.

Result: There were no deaths following treatment.

During the first week post dosing lacrimation and a discharge covering the

perineum (genital area) were observed.

At termination of the study all animals appeared healthy and had gained

weight.

The oral LD_{50} was judged to be greater than 5 g/kg.

Reliability : (2) valid with restrictions

Not clear whether this study was carried out to GLP and few experimental data given. Nevertheless, the study is sufficient to demonstrate an LD₅₀ of

greater than 5 g/kg.

(55)

Type : LD_{50}

Result: The results of other acute toxicity studies which have been reported are as

follows:

Sample LD₅₀ Clinical signs

Lost Hills Light (Mobil consolidated report)

>5 g/kg Soft stool

Urogenital discharge
Decreased fecal output

Anal discharge

MCSL Crude (midcontinent) Mobil study No. 40971

>5 g/kg Decreased activity

Hunching

Discharge/perineal staining

Arab Light (Mobil study No. 40961)

>5 g/kg Discharge/perineal staining

Belridge heavy (Mobil summarized data)

>5 g/kg Mild gastrointestinal

effects

Smith et al (1981) also demonstrated that three different crude oils (Crude

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type not specified) were non-toxic in male mice as follows:

Wilmington Crude oil >16 g/kg Recluse crude oil >16 g/kg Mixed petroleum crudes >10 g/kg

(54) (56) (63) (73) (76)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}

Value : > 2000 mg/kg bw

Species : rabbit

Strain : New Zealand white

Sex : male/female

Number of animals : 3

Vehicle : None, undiluted

Doses : Single dose: 2000 g/kg

Year : 1984 GLP : no data

Test substance: Beryl Crude (Light crude oil)

Method: The skin was clipped from the trunks of three male and three female New

Zealand White rabbits prior to treatment with test material. The skin of the backs of three animals (2 male, 1 female) was abraded and the skin of the remaining animals was left intact. The liquid, undiluted test material was then applied as a single dose of 2 g/kg to the shorn back of all six animals. The test site was covered with a gauze and an occlusive wrap. The animals were also fitted with Elizabethan collars to prevent chewing of the occlusive covering and ingestion of test material. 24 hours after application of the test material the occlusive dressing was removed and any surplus test material was removed from the skin by gently wiping with cotton moistened with physiological saline. The animals were observed frequently

on the day of treatment and daily thereafter for 14 days.

Two hours after wiping the residual material from the skin (26 hours after treatment), the areas of application were assessed for skin irritation. Further evaluations for skin irritation were also made on the 3rd and 7th

days following treatment.

Result: There were no deaths in this study.

A few animals had soft stool/diarrhea during the observation period, however, all animals were normal at study termination. At the end of the study five of the six animals had gained weight and one had lost a small amount of weight. [No actual body weight data are provided in the report]. There was no evidence of systemic toxicity during the study although there

was some skin reaction at the site of application.

Following 24 hours of skin contact, varying degrees (slight, moderate) irritation were observed at the 26 and 72 hour readings. At the end of the

first week, the skin response was barely perceptible or absent.

The dermal LD_{50} was judged to be greater than 2 g/kg

Reliability : (2) valid with restrictions

Not clear whether this study was carried out to GLP and few experimental

data given.

(52)

Date: November 15, 2003

Type : LD_{50}

Result : Additionally, acute dermal LD₅₀s have also been reported for three other

light crude oils.

The results are as follows:

Sample LD₅₀ Clinical signs

Lost Hills Light (Mobil Study No. 63831)

>2 g/kg Soft stool

Decreased food consumption Decreased fecal output

MCSL Crude (midcontinent) Mobil study No. 40972

>2 g/kg Diarrhea

Nasal discharge

Arab Light (Mobil study No. 40962)

>2 g/kg No signs of toxicity

Belridge Heavy (Mobil summarized data)

>2 g/kg Mild gastrointestinal effects

(51) (53) (63) (73)

5.2.1 SKIN IRRITATION

Species: rabbitConcentration: undilutedExposure: OcclusiveExposure time: 24 hour(s)

Number of animals : 6

Vehicle : Undiluted Year : 1985 GLP : no data

Test substance : Lost Hills Light Crude

Method : Prior to test the hair was clipped from the backs of six New Zealand White

rabbits (sex not specified). Three one inch square sites (anterior, middorsal and posterior) on the right side of each animal were lightly abraded. Three similarly orientated sites were left intact on the left side of the animal,

thus providing six test sites on each rabbit.

0.5 ml of test material was applied to each test site. The material applied to the anterior and mid-dorsal sites was covered with a Webril patch and these were occluded. The dorsal sites remained open. The rabbits were fitted with collars to prevent ingestion of the test material and chewing of

the occluded patches.

Four hours after treatment, the anterior sites were unwrapped. The intact site was immediately examined for evidence of corrosive effects and both sites were then gently wiped with a gauze. Thirty minutes later, the skin was evaluated for irritation. The sites were evaluated again 24 hours after patch removal. 48 hours after application, the 4 hour occluded intact site was re assessed for corrosion. Both sites were scored for irritation 4 hours later. The 4 hour occluded sites were again re-evaluated for irritation on

day 3.

24 hours after treatment, the two mid-dorsal sites were unwrapped and

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along with the posterior uncovered sites were wiped with cotton. The four sites were evaluated for irritation 2 hours later, again approximately 72 hours later and finally on the 7th day after treatment.

Result

: 4 Hour occluded sites (DOT, OECD methods)

Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively.

The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites.

Actual scores were:

	Intact		Abraded	
	Erythema	Edema	Erythema	<u>Edema</u>
4.5 hrs	1.0	1.2	1.0	1.3
28 hrs	1.2	1.0	1.0	1.0
52 hrs	1.7	1.3	1.7	1.3
76 hrs	1.8	1.5	1.8	1.7
7 days	0.8	0.7	0.5	0.7
10 days	0	0.3	0	0

24 Hour occluded sites (FHSA method)

Actual scores were:

	Intact		Abraded		
	Erythema	Edema	Erythema	<u>Edema</u>	
26 hrs	1.7	1.7	1.7	1.7	
72 hrs	2.3	1.5	2.2	1.7	
7 days	1.2	0.7	0.8	0.8	
10 days	0.2	0	0.2	0.2	

Non-occluded sites

Actual scores were:

	Intact		Abraded	
	Erythema	Edema	Erythema	<u>Edema</u>
26 hrs	1.7	1.2	1.8	1.3
72 hrs	2.8	1.5	2.7	1.3
7 days	1.3	0.8	1.3	8.0
10 days	0.3	0	0.2	0

Conclusion

: The classification according to various methods/criteria are summarized as follows:

Guideline	Score	Rating
DOT Corrosion EEC (4hr occluded)	Negative Erythema 1.6 Edema 1.3	Non-corrosive Non-irritant
OSHA PII (4 h occl.) FHSA PII (24 h occl.) (1) valid without restrict	2.8 3.6 etion	Non-irritant Non-irritant

Reliability

(63)

Date: November 15, 2003

Species: rabbitConcentration: undilutedExposure: OcclusiveExposure time: 24 hour(s)

Number of animals : 6

Vehicle : Non, undiluted

Year : 1984 GLP : no data

Test substance : Arab Light crude

Method

Prior to test the hair was clipped from the backs of six New Zealand White rabbits (sex not specified). Three one inch square sites (anterior, middorsal and posterior) on the right side of each animal were lightly abraded. Three similarly orientated sites were left intact on the left side of the animal, thus providing six test sites on each rabbit.

0.5 ml of test material was applied to each test site. The material applied to the anterior and mid-dorsal sites was covered with a Webril patch and these were occluded. The dorsal sites remained open. The rabbits were fitted with collars to prevent ingestion of the test material and chewing of the occluded patches.

Four hours after treatment, the anterior sites were unwrapped. The intact site was immediately examined for evidence of corrosive effects and both sites were then gently wiped with a gauze. Thirty minutes later, the skin was evaluated for irritation. The sites were evaluated again 24 hours after patch removal. 48 hours after application, the 4 hour occluded intact site was re assessed for corrosion. Both sites were scored for irritation 4 hours later. The 4 hour occluded sites were again re-evaluated for irritation on day 3.

24 hours after treatment, the two mid-dorsal sites were unwrapped and along with the posterior uncovered sites were wiped with cotton. The four sites were evaluated for irritation 2 hours later, again approximately 72 hours later and finally on the 7th day after treatment.

Result : 4 Hour Occluded (OECD Method, DOT)

Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 0.9 and 0.1 respectively.

The initial response of the skin to the test material was slight. Within 72 hours after treatment, most sites had recovered, with little difference in response between intact or abraded sites.

24 Hour Occluded (FHSA method)

The day following application of the test material a moderate erythema was observed. This subsided during the subsequent days such that there was only barely perceptible irritation at the end of the first week. Abrasion had no effect on either the initial response or the recovery.

Non Occluded

Uncovered treated sites had moderate erythema 24 hours after application of the test material. This response diminished and was virtually absent after one week.

Abrasion had no effect on either the initial response or the recovery.

Reliability : (2) valid with restrictions

A textual description of the results only was available. No actual data were provided in the report.

(60)

Date: November 15, 2003

Species : rabbit

Concentration : undiluted

Exposure : Occlusive

Exposure time : 4 hour(s)

PDII : 1.9

Result : not irritatin

Result : not irritating
Classification : not irritating
Year : 1997

Test substance : Belridge heavy crude

Result : Summarized data for Belridge Heavy crude oil are:

Guideline	Score	Rating				
DOT Corrosion EEC (4hr occluded)	Negative Erythema 0.6 Edema 0.8	Non-corrosive Non-irritant				
OSHA PII (4 h occl.)	1.4	Non-irritant				
FHSA PII (24 h occl.)		Non-irritant				
(2) valid with restrictions						
Data only available in	Data only available in tabular form, but studies were part of a series of					

studies using same methods, all of which were reliability 1.

(73)

5.2.2 EYE IRRITATION

Reliability

Species: rabbitConcentration: undilutedDose: .1 mlComment: not rinsedNumber of animals: 6

Year : 1985

Test substance : Beryl Crude (Light crude oil)

Method : 0.1 ml of undiluted test material was instilled into one eye of each of six

New Zealand White rabbits. The eyes were not washed and were evaluated for irritation at 1, 24, 48 and 72 hours after treatment.

Fluorescein was used to aid the evaluation of the 72 hour reading. Treated eyes that stained positively with fluorescein were examined again 7 and 14

days after treatment.

Result: The mean irritation scores at each of the observation times were:

	<u> 1 hr</u>	24 hr	<u>48 hr</u>	<u>72 hr</u>
Cornea	0	0	0	0
Iris	0	0	0	0
Conjunctivae	4.0	1.7	1.3	1.0

The test material was judged to be non-irritating to the rabbit eye.

Reliability : (2) valid with restrictions

The only reservation is that it is not clear whether this study was carried out

according to GLP. Otherwise the study and reporting are sound.

(58)

Date: November 15, 2003

Species : rabbit

Result : Eye irritation studies have been reported for three other samples of light

crude oil.

There were no effects on either the iris or cornea. The only effects

recorded were on the conjunctivae.

The mean irritation scores (conjunctivae only) are as follows:

Crude type	Result				Ref
	1 hr	24 hr	48 hr	72 hr	
MCSL (Midcontinent)					
	2.3	0.3	0	0.7	Mobil 40973
Loot Hillo Light					
Lost Hills Light	8.0	3.7	2.7	1.7	Mobil 63832
	0.0	0		•••	
Arab Light					
	5.3	1.3	0.7	0.3	Mobil 40963
Belridge Heavy	0.9	8.0	0.9	Mobil 1	1997
-					(57) (59) (63) (73

5.3 SENSITIZATION

Type : Buehler Test Species : guinea pig

Concentration : 1st: Induction 15 % occlusive epicutaneous

2nd: Challenge 15 % occlusive epicutaneous 3rd: Challenge 10 % occlusive epicutaneous

Number of animals : 20

Vehicle : Mineral oil Result : not sensitizing

Year : 1991 **GLP** : yes

Test substance : Lost Hills Light Crude

Method : Concentrations of test material used in this sensitization study were

determined in a pre screening study.

Induction phase

0.4 ml of a 15% w/w concentration of test material in mineral oil was applied using a Hill Top Chamber with a 25 mm Webril swatch to the shorn backs of 10 male and 10 female Guinea pigs. The patch was occluded for six hours. The patches were applied to the same site once per week for 3 weeks. After each 6-hour exposure period, the patches were removed and the skin wiped with gauze moistened with mineral oil. The positive control material (DNCB) was applied at a concentration of 0.05% w/w in 70% ethanol to 5 male and 5 female Guinea pigs. Treatment of the positive control animals was the same as for the test animals, except that the skin was wiped with saline after patch removal.

Challenge

Challenge patch application was performed 14 days after the last induction dose had been applied. Dual challenge patches were applied to fresh application sites of previously shorn skin of the animals. The test material

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was applied at concentrations of 10 and 15% w/w in mineral oil. The patches were then occluded for 6 hours.

On the day following challenge patch application, the skin was depilated and 2 hours later and scored for signs of sensitization. The sites were examined after a further 48 hours but this time without depilation. Naive and positive control animals were challenged with DNCB at a

concentration of 0.05% in acetone.

Result : The results were as follows:

	No.	Mean erythema
	responding	score
Test material (10% concentre	ration)	
24 hrs induced	3/19	0.2
24 hrs control	0/10	0
48 hrs induced	3/19	0.3
48 hrs control	2/10	0.2
Test material (15% concentr	ration)	
24 hrs induced	2/19	0.2
24 hrs control	2/10	0.2
48 hrs induced	1/19	0.1
48 hrs control	1/10	0.1
DNCB positive control		
24 hrs induced	5/5	3.4
24 hrs control	0/5	0
48 hrs induced	5/5	2.6
48 hrs control	0/5	0

Although two test animals responded, the test material was judged to be non-sensitizing, since the test criterion required an mean erythema score of +2 for a positive response.

Reliability : (1) valid without restriction

(66)

Type : Buehler Test

Concentration : 1st: Induction 15 %

2nd: Challenge 10 % 3rd: Challenge 15 %

Result : not sensitizing

Year : 1991

Test substance: Belridge heavy crude

Result: A similar Buehler test of Belridge heavy crude oil was also negative.

Reliability : (2) valid with restrictions

(65)

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REPEATED DOSE TOXICITY

: Sub-chronic **Type**

Species rat

Sex male/female Strain Sprague-Dawley

Route of admin. dermal

Frequency of treatm. 5 days/week for 13 weeks 30, 125 and 500 mg/kg/day Doses Control group ves, concurrent no treatment

Year 1992 **GLP** no data **Test substance** Two crude oils

Method

Two separate but identical studies are reported, one for each of two crude oils (Crude I and Crude II). The methods used were identical for both studies.

Undiluted test material was applied to the shorn unoccluded skin of groups of ten male and ten female Sprague-Dawley rats at doses of 30, 125 and 500 mg/kg/day. Application was once daily, five times each week for 13 weeks. Groups of ten rats of each sex served as untreated controls. Each animal was fitted with an Elizabethan collar to prevent ingestion of the applied test material. At the end of each week residual test material was wiped from the backs of the animals. Animals were observed regularly for clinical signs of toxicity and body weights were recorded weekly. The animals were sacrificed during week 14 of the study after fasting overnight. All animals were necropsied and blood samples were taken for a range of hematological and serum chemistry determinations. A range of organs were weighed and tissues examined histologically.

An additional two groups of ten males were treated at a dose level of 0 and

500 mg/kg/day for an evaluation of male reproductive health.

For these animals testes weight and cauda epididymis weights were recorded. Additionally, the number of sperm and % normal sperm in the cauda were recorded as well as the number of spermatids in the testis.

Result

No animals died in either of the studies and there were no clinical signs of systemic toxicity attributable to either crude oil. Both crude oils caused the same minimal skin irritation (flaking) at the exposure site.

Body weights

Apart from the 500 mg/kg/day group for Crude II that gained less weight than the controls (217g compared to 247g over the course of the study). All other body weight measurements were similar to the respective controls.

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Hematology

Significant changes relative to their respective controls only occurred in the highest dose group animals (500 mg/kg/day). The % increases (+) or decreases (-) that occurred in these dose groups are shown in the following table. [Changes are shown to the nearest whole number]

Parameter	Crude I		Crude II	
	Males	Females	Males	Females
RBC	-6%	NSD	-8%	-7%
Hemoglobin	-6%	NSD	-11%	-8%
Hematocrit	-5%	NSD	-9%	-8%
Platelets	NSD	NSD	-19%	NSD

Doso group (malkaldov)

NSD No significant difference

Serum chemistry

Although more parameters were affected in the high dose Crude II females, there was no consistent pattern of change that could be attributed to the two crude oils. The differences %+ or %- are shown in the table below.

Dose group (mg/kg/day)						
	Crude	l		Crude	II	
	30	125	500	30	125	<u>500</u>
MALES Calcium Glucose Urea nitrogen Uric acid		+13%	-7% +13% +53%			-27%
FEMALES ALT Cholesterol Glucose Potassium Urea nitrogen Uric acid	+16%		+20% -11%		+41%	-18% +60% -13% +31% -27%

Necropsy findings

There were no treatment-related observations at necropsy. With the exception of the liver and thymus, there were no absolute or relative organ weight changes when compared to controls.

The organ weight differences that were observed were confined to the 125 and 500 mg/kg/day groups and are summarized below.

Organ	1	Crude	e I	Crude	II
wt		125	500	125	<u>500</u>
MALE	<u>S</u>				
Liver	(absolute)		+20%		+22%
	(relative)		+22%	+18%	+33%
Thymu	us (absolute)				-41%
FEMA	<u>LES</u>				
Liver	(absolute)		+13%		+24%
	(relative)		+12%		+31%
Thymu	us (absolute)				-35%
	(relative)				-27%

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Histopathology

Changes in the skin included hyperplasia and an associated dermal inflammatory cell infiltration. In general the effects were slightly more severe in the animals treated with Crude I. All male and female animals exposed to Crude I were affected whereas at the lowest dose level 8/10 male and 6/10 females exposed to Crude II were affected.

Other histopathological findings were associated with the bone marrow, Liver, thymus and thyroid although effects generally were greater with crude II than with crude I. The findings are tabulated below

Crude I

Bone marrow No effects in either sex at any dose level

Liver Multifocal, mononuclear cell infiltration in 3 males and 2

females at 500 mg/kg/day.

Multifocal hepatocellular vacuolation in 3 females at 500

mg/kg/day only

Thymus Atrophy in one male and two females at 500mg/kg/day only

Thyroid Hypertrophy and hyperplasia of follicular epithelium in some

males and females at all dose levels

Crude II

Bone marrow Increased cellularity in 2 males at each dose level (6 males in highest dose group) and focal necrosis in 2

males in the 50 mg/kg/day group.

In the females increased cellularity was observed in 9/10

animals

Liver Hepatocellular vacuolation in one male and one female in

the 500 mg/kg/day group.

Mononuclear cell infiltration was also observed but in only

one male in the highest dose group

Thymus Atrophy was observed in six males and 7 females in the

500 mg/g/day group.

Thyroid Hypertrophy and hyperplasia was seen in a few animals at

all dose levels.

The spermatozoa/spermatid evaluations that were conducted on the separate 500 mg/kg/day group of animals exposed to crude I did not reveal any effects when compared to a control.

Test substance

Two crude oils were examined in the paper by Feuston et al The characteristics of the crude oils was reported as:

Chemical class	Crude	<u> ICrude II</u>
Nonaromatics	50.0	37.3
<3-Ring PAH	35.3	41.7
3- to 5- Ring PAH	10.2	15.7
3- Ring PAH	5.9	8.1
4- Ring PAH	2.2	4.0
5- Ring PAH	2.1	3.6
Sulfur-PAC	2.4	2.9

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Nitrogen (N)-PAC 5.4 8.4 Nonbasic N-PAC 3.8 5.9

Total 103.3 106.0

From information available in the Mobil report of study No 63834 and other laboratory data, it is clear that Crude I is Lost Hills Light Crude and Crude II is Belridge heavy crude oil.

(30)(72)

Type : Sub-acute
Species : mouse
Sex : male/female

Strain: CD-1Route of admin.: gavageExposure period: 5 days

Frequency of treatm. : Daily for five days

Doses : 2, 4, 8, 12 & 16 ml/kg/day Control group : yes, concurrent no treatment

Year : 1990 GLP : no data

Test substance : Prudhoe Bay Crude oil (Heavy crude oil)

Method: Two pilot studies were conducted with groups of 5 male and 5 female mice.

In these studies Prudhoe Bay crude oil was administered by gavage at doses of 0, 5 and 10 ml/kg daily for five days. No other experimental details are given. However, the results led the author to conduct four

further follow-up studies and these are described below.

Experiment 1 (Three crudes, each at a single dose level)

One of three crude oils or Bunker C oil was administered by gavage, daily at a single dose level of 10 ml/kg for 5 days to groups of 10 male mice. A further group of 10 male mice were intubated each day but no material was administered: these animals served as controls.

Experiment 2 (One crude, single dose level)

Groups of 10 male mice were given either Prudhoe Bay crude oil or mineral oil USP or corn oil at a dose of 10 ml/kg/day for five days. A separate group of 10 controls were intubated but no material was administered. This study was to compare the effect of one crude oil with the effects of two oils expected to be non-toxic.

Experiment 3 (One crude, five dose levels)

Prudhoe bay crude oil was administered to 10 or 11 male mice at dose levels of 2, 4, 8, 12 or 16 ml/kg/day. A further group of 10 male mice were sham treated and served as controls.

A fourth experiment was also carried out which studied the effect of crude oil exposure on vitamin E and selenium-deficient animals. This study is not summarized here.

In all experiments 1-3 the animals were weighed on the day they were first dosed and again prior to necropsy. The differences in weights were recorded as change in body weight during the studies. Necropsy was 24 hours after the 5th dose had been administered.

At necropsy, blood samples were taken for determination of blood cell counts, packed cell volume (PCV), hemoglobin and red cell indices

5. Toxicity

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(MCHC). Blood smears were stained with methylene blue to detect the presence of Heinz bodies in red cells. Liver was removed and weighed whereas the spleen and thymus were weighed after fixation. Portions of all major organs were fixed for subsequent histological examination.

Data were analyzed by analysis of variance and regression procedures of the SAS programs (SAS Institute Inc., Cary, NC)

: Although several studies were reported, the only studies summarized here are study A in which several crude oils are tested at a single dose level and study C in which PBCO is examined at five different dose levels. Neither information on oils other than crude oil or on the effect of Vitamin E and selenium-depletion are included in this robust summary.

In the pilot studies it was reported that the oils were distasteful to the mice and that after the first dose, administration of test material was difficult. Several mice from each group died from inhalation of oil that occurred during the oral dosing. The author stated that the pilot studies demonstrated a dose related decrease (8-11%) in PCV, a reduction in body weight gain, a 74% increase in liver weight per unit body weight and a 66% reduction in thymus weight. No data are given but this was the reason given for the further studies that were carried out.

Although the studies carried out were separate, the author reported the results together and these are summarized below.

Hematology

It was reported (but no data presented) that in experiment A none of the oils tested resulted in significant changes in PCV, number of red blood cells or whole blood hemoglobin. The following table summarizes the hematological results obtained with PBCO only.

Study	No of	Daily dose PBCO	PCV	MCHC
	mice	(ml/kg/day)		(g/l)
Α	10 8	0 10	0.43±0.02 0.42±0.04	336±22 328±10
С	11 8	0 12	0.43±0.04 0.43±0.02 0.40±0.03	328±11 348±3*

^{*} p<0.05, analysis of variance with Tukey's multiple range procedure

Body and organ weights

The organ and body weight changes are summarized in the following tables

Study A comparison of three different crude oils

Treatment group					
Control	PBCO	SLCO	ALCO		
Group size					
10	8	6	7		
Change in body wt (g) +1.74±0.88	-0.33±1.5	-0.77±2.36*	-1.43±2.28*		
Liver wt (g) 2.15±0.2	3.03±0.54*	2.73±0.5	2.66±0.43*		

Result

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Liver/body wt (g/g) 0.07±0	0.11±0.01*	0.10±0.01*	0.09±0.01*
Spleen/body wt (g/g) 3.52±0.61	2.46±0.24*	2.49±0.29*	2.17±0.66*
Thymus/body wt (g/g) 2.26±0.46	0.85±0.33*	1.01±0.41*	1.12±0.46*

^{*} P<0.05 Analysis of variance with Tukey's multiple range procedure

Study C: PBCO at 5 dose levels

Body wt change (g)	Liver wt (g)	Organ/liver	body wt (g/g) spleen	thymus
Control group (+3.14±1.33	(10 mice) 1.95±.18	.06±.01	3.9±.49	2.7±.29
2 ml/kg/day (10 -0.88±2.57		.09±0	2.65±.62	1.61±.49*
4 ml/kg/day (9 -0.86±1.24	,	.09±.01*	2.57±.23*	1.35±.32*
8 ml/kg/day (8 -1.66±2.03*	,	.1±.01*	2.31±.67*	1.03±.4*
12 ml/kg/day (9 -1.66±1.71*		.1±.01*	2.2±.44*	.71±.22*
16 ml/kg/day (7 -3.29±1.4*	,	.1±.01*	2.26±.87*	.65±.15*
Coefficient of c	letermination (Ri	²) 0.48***	0.31***	0.64***

P<0.05 Analysis of variance with Tukey's multiple range procedure

There were no gross abnormalities at necropsy except a reduction in the size of thymus glands in those animals that had received crude oil. Liver, kidney, spleen, lung and thymus from groups of 5 mice that had received 0, 5 or 10 ml PBCO for 5 days in the pilot study were examined histologically. The thymus glands of mice in the 10 ml/kg/day group had very thin cortices and reduced densities of lymphocytes in the remaining cortex compared to controls. Hepatocytes from these same animals had uniformly dense cytoplasm while control mouse hepatocytes had large areas of rarified cytoplasm typical of normal glycogen-replete mouse livers. The histology of the thymus glands of the 5 ml/kg/day group was intermediate between the controls and the 10 ml/kg/day group.

There were no histological lesions in the other tissues examined. Overall, the results demonstrated minor hematological change, liver enlargement and atrophy of spleen and thymus.

: The following crude oils were examined:

Test substance

P<0.03 Analysis of variance in regression

^{***} P<0.001 Analysis of variance in regression

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Prudhoe Bay crude oil (PBCO) [a heavy crude] South Louisiana crude oil (SLCO) [a light crude]

Arabian Light crude oil (ALCO)

In addition a bunker C oil was examined

A USP grade mineral oil and a corn oil were also included in the studies.

Reliability : (2) valid with restrictions

These studies were probably not carried out according to GLP and the studies did not include measurement of all the parameters normally measured in repeat dose studies. Although the results are of limited value they do nevertheless add some information on the effect of the repeated

exposure of mice to crude oil.

(41)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Modified Ames Assay

System of testing: Ames test of a DMSO extract of test substance

Test concentration : 1 to 50µl/plate

Metabolic activation: withYear: 1984GLP: no data

Test substance : Beryl Crude (Light crude oil)

Method : DMSO extracts were prepared by mixing 2 ml test material with 3 ml

cyclohexane to homogeneity. 10 ml DMSO was then added and mixed thoroughly. The mixture was vortexed every 5 minutes for a total of 30 minutes. After 30 minutes the mixture was centrifuged and the DMSO

layer (extract) removed and stored for testing.

The extract was only tested with metabolic activation in Salmonella typhimurium strain TA 98 at the following doses: 1, 3, 5, 7, 10, 15, 25 and 50 μ l/plate. Additionally the DMSO extract of a carcinogenic oil was also tested in the same manner.

Positive control chemicals were: 2-aminoanthracene (2 µg)and benzoa(a)pyrene (5 µg).

The DMSO extract of a refrigerator oil was used as negative control (50 μ l). The metabolic activation mixture was derived from Araclor-induced hamster liver and this was use at eight times the standard concentration (0.4 ml rather than 0.05 ml).

The test material and control substances were added to tubes at the doses shown above and were incubated for 20 minutes with Salmonella broth culture. Colonies of histidine prototrophs were counted 48 hours after plate incubation.

Assay acceptance criteria

A linear dose response curve for mutagenicity must be obtained before the test material can be ranked for potency

Spontaneous and solvent control reversion rates for TA98 must fall between 20 and 50 revertants /plate $\,$

The slope of the dose response curve for the positive control carcinogenic oil must fall between 1.5 - 4.5 netrevertants/plate

Provided the above criteria are met the net revertants per plate is plotted

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against dose and the best initial straight line response is determined by regression analysis. Test materials producing slopes less than 0.5 net

revertants/µl are considered inactive.

Result : The No. revertants for each dose level of Beryl crude are as follows (NB

data for the carcinogenic oil are not shown):

Dose (µI)	No. of revertants
0 (Spontaneous)	43
0 (solvent control)	39
50	113
25	85
15	74
10	67
7	61
5	57
3	47
1	48
2-AA control	540
BaP control	366
Refrigerator oil 43	

The slope for the Beryl crude was determined to be 2.5

The slope for the carcinogenic oil was 3.7

Remark: Similar studies were conducted for Arab light, MCSL, Lost Hills light and

Belridge Heavy crudes.

The results were:

Crude	Slope	Reference
Arab light	3.8	Mobil study 40965
MCSL crude	1.5	Mobil study 40975
Belridge Heavy	1.7	Mobil study 663850
Lost Hills light	0	Mobil study 63838

Conclusion: A modified Ames assay conducted on five different crudes demonstrated

that all but one (Lost Hills light) were mutagenic. The Lost Hills Light did

not demonstrate any mutagenic activity.

Reliability : (2) valid with restrictions

It is not clear whether the studies were carried out according to GLP. Furthermore, the assay was designed as a screen for carcinogenic activity However, the results are useful to identify the mutagenic potential of crude

oils.

(48) (49) (50) (61) (62)

Type : Cytogenetic assay

System of testing : Chinese Hamster Ovary cells

Test concentration : 1 to 20 µl DMSO extract/ml culture medium

Metabolic activation: withResult: negativeYear: 1991GLP: no data

Test substance : Lost Hills Light crude

Method : Preparation of DMSO extract

10 ml DMSO was added to approximately 2 ml test substance. The tube containing the mixture was vortexed every 5 minutes for one

minute. After 30 minutes, the mixture was centrifuged and the DMSO layer

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(Extract) was removed for testing.

Control substances:

Solvent control: DMSO

Negative control: Untreated flasks

Positive controls: Cyclophosphamide monohydrate at concentration

of 10 µg/ml

Duplicate cultures were used - one set for metaphase analysis and the other for a determination of cytotoxicity (Mitotic Index). All cultures were conducted with metabolic activation.

CHO cells were exposed for two hours to test material at concentrations of 1, 2.5, 5, 10, 15 and 20 μ l/ml. After two hours, treatment was terminated and the cultured cells were washed and re-fed with complete culture medium. Approximately 16 hours later, colcicine was added and two hours later the cells were harvested as described below.

Metaphase analysis

Metaphase cells were collected by tapping a flask to release the loosely attached mitotic cells into culture medium and pelleting them by centrifugation. The cells were resuspended and then fixed in methanol:acetic acid (3:1) and stored refrigerated until slide preparation. A minimum of 4 slides was made for each culture flask. 100 cells were examined microscopically per flask and were scored for structural chromosomal aberrations. Although gaps were recorded they were not used in the analysis for chromosomal aberration since their significance is questionable.

Cytotoxicity assay (Mitotic Index determination)

For this portion of the assay all cells in each flask were collected. The medium in each flask was replaced and 5 ml of trypsin was added and after incubation for approximately 5 minutes the flasks were examined to ensure that the cells had rounded up. The cells were then released into the trypsin solution mechanically and the resulting suspension was centrifuged in the presence of medium. The centrifuged cells were swelled in hypotonic solution and were fixed in methanol:acetic acid as above. A minimum of 2 slides per flask were prepared for the determination of mitotic index. The slides were stained with Giemsa and at least 1000 nuclei per flask were scored as either mitotic or interphasic.

The MI = No mitotic cells x 100
Total No of cells scored

Criteria for a positive or negative response

A test substance is considered to have elicited a positive response if at least one concentration shows a statistically significant increase in the proportion of cells with aberrations and a significant positive dose-response exists.

Biological significance of the result is also taken into account in determining the response.

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Result : Determination of Mitotic Index (MI)

There was not a dose related decrease in MI at treatments that were not toxic. The MI at each of the dose concentrations of Lost Hills Light crude (LHL)was:

Treatment	Mitotic Index
20 µl/ml extract of LHL	no cells found
15 µl/ml extract of LHL	no cells found
10 µl/ml extract of LHL	0.6
5 µl/ml extract of LHL	10.7
2.5 µl/ml extract of LHL	8.4
1 µl/ml extract of LHL	12.2
20 μl/ml DMSO	7.6
Negative control	10.4

Metaphase analysis

Treatment

The concentrations analyzed for chromosomal aberrations were 5, 2.5 and 1.0 µg/ml.

No of cells with

There was no increase in the proportion of cells with structural chromosome aberrations (gaps excluded). Nor was a dose response observed. The data are:

out.iioiii	one or more aberrations (100 cells examined)
5 µl/ml extract of LHL	2
2.5 µl/ml extract of LHL2	
1 µl/ml extract of LHL	1
DMSO (solvent control	2
Negative control	2
CP (positive control)	28
(4) Ital Ital t	

Reliability

: (1) valid without restriction

Despite the fact that whether the study was carried out to GLP is not clear,

it is, nevertheless, reliable and valid.

(70)

Type Cytogenetic assay

System of testing Chinese Hamster Ovary cells

Test concentration 1 to 20 µl DMSO extract/ml culture medium

Metabolic activation with Result negative Year : 1991 **GLP** : no data

Test substance : Belridge Heavy crude

Preparation of DMSO extract Method

10 ml DMSO was added to approximately 2 ml test substance. The tube containing the mixture was vortexed every 5 minutes for one minute. After 30 minutes, the mixture was centrifuged and the DMSO layer (Extract) was

removed for testing.

Control substances:

Solvent control: **DMSO**

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Negative control: Untreated flasks

Positive controls: Cyclophosphamide monohydrate at

concentration of 10 µg/ml

Duplicate cultures were used - one set for metaphase analysis and the other for a determination of cytotoxicity (Mitotic Index). All cultures were conducted with metabolic activation.

CHO cells were exposed for two hours to test material at concentrations of 1, 2.5, 5, 10, 15 and 20 μ l/ml. After two hours, treatment was terminated and the cultured cells were washed and re-fed with complete culture medium. Approximately 16 hours later, colcicine was added and two hours later the cells were harvested as described below.

Metaphase analysis

Metaphase cells were collected by tapping a flask to release the loosely attached mitotic cells into culture medium and pelleting them by centrifugation. The cells were resuspended and then fixed in methanol:acetic acid (3:1) and stored refrigerated until slide preparation. A minimum of 4 slides was made for each culture flask. 100 cells were examined microscopically per flask and were scored for structural chromosomal aberrations. Although gaps were recorded they were not used in the analysis for chromosomal aberration since their significance is questionable.

Cytotoxicity assay (Mitotic Index determination)

For this portion of the assay all cells in each flask were collected. The medium in each flask was replaced and 5 ml of trypsin was added and after incubation for approximately 5 minutes the flasks were examined to ensure that the cells had rounded up. The cells were then released into the trypsin solution mechanically and the resulting suspension was centrifuged in the presence of medium. The centrifuged cells were swelled in hypotonic solution and were fixed in methanol:acetic acid as above. A minimum of 2 slides per flask were prepared for the determination of mitotic index. The slides were stained with Giemsa and at least 1000 nuclei per flask were scored as either mitotic or interphasic.

The MI = No mitotic cells x 100
Total No of cells scored

Criteria for a positive or negative response

A test substance is considered to have elicited a positive response if at least one concentration shows a statistically significant increase in the proportion of cells with aberrations and a significant positive dose-response exists. Biological significance of the result is also taken into account in determining the result.

Determination of Mitotic Index (MI)

There was not a dose related decrease in MI at treatments that were not toxic. The MI at each of the dose concentrations of Belridge Heavy crude (BH)was:

Treatment	Mitotic Index
20 µl/ml extract of BH	no cells found
15 µl/ml extract of BH	no cells found
10 µl/ml extract of BH	no cells found
5 μl/ml extract of BH	11.2
2.5 µl/ml extract of BH	10.2

Result

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13.1 1 µl/ml extract of BH 20 µl/ml DMSO 7.6 Negative control 10.4

Metaphase analysis

The concentrations analyzed for chromosomal aberrations were 5, 2.5 and 1.0 µg/ml. There was no increase in the proportion of cells with structural chromosome aberrations (gaps excluded). Nor was a dose response observed. The data are:

Treatment	No of cells with one or more aberrations (100 cells examined)
5 μl/ml extract of LHL	1
2.5 µl/ml extract of LHL	2
1 µl/ml extract of LHL	1
DMSO (solvent control	2
Negative control	2
CP (positive control)	28
(1) valid without roofriction	

Reliability : (1) valid without restriction

Despite the fact that whether the study was carried out to GLP is not clear,

it is, nevertheless, reliable and valid.

(69)

Type Metabolic activation

with and without

Year 1981 **GLP** : no data

Test substance : South Louisiana crude (Light crude oil)

Ames test

Method : The authors summarized the protocol in the following table:

Test strains

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100

Liver homogenate

S-9 from male Charles River CD rat liver 500 mg Aroclor/kg for 5 days

Procedure

Treatment without activation: 2 ml top agar, 0.1 ml dissolved test chemical, 0.1 ml bacterial culture (108 cells)

Treatment with activation: 2 ml top agar, 0.1 ml dissolved test chemical, 0.1 ml bacterial culture (10⁸ cells) plus 0.5 ml S-9 mix contains per milliliter 0.3 ml S-9 (1 g tissue + 3 ml 0.15M KCl), 8 mM Mg Cl₂, 33 mM KCl, 5mM glucose-6-phosphate, 4 mM NADP, and 100 mM sodium phosphate (pH 7.4).

Incubation period: 48 hr

Preliminary toxicity determination Design:

Duplicate plates/point

Five test concentrations and solvent and positive controls

Duplicate experiments

Solvent Dimethyl sulfoxide

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Data analysis

Mutagenic: statistically significant. Increase in total revertant colony number (P= 0.01) and dose response (P= 0.01)

Scorina

<u></u>	Revertant/µg	Induced frequency/ Spontaneous frequency	
·)	<0.01	_*	
+)	0.1 to 0.01	<5	
++)	>0.1	>5	

* No greater than response

Result The results are given in the following table

Sample	Muta (wit)			
	TA-1537	TA-1538	TA-98	TA-100
Benzo[a]pyrene	++	++	++	++
South Louisiana crude	-	-	-	+

It is not clear from the report whether any activity was detected in the assay

without metabolic activation.

Reliability : (4) not assignable

The study is not fully reported (data for the assay without

metabolic activation are not given)

(9)

Ames test Type Metabolic activation with and without negative Result

1982 Year **GLP** : no data

Test substance : Wilmington crude

Method Salmonella typhimurium strains TA 98 and TA 100 were used in this study.

> The test sample was suspended in Tween 80 prior to testing. Each sample was weighed in a glass vial and then an equal weight of Tween 80 was added. The oil and detergent were stirred until thoroughly homogenized. Distilled water was then added dropwise with continuous stirring until a stable, homogenous suspension was obtained. The concentration of the mixture was then adjusted to 10% sample - 10% Tween 80 (w/v) by the

addition of more distilled water.

The assay was carried out in triplicate with and without metabolic activation by S9 liver homogenate that was prepared from Aroclor-induced Sprague-Dawley rats. The S9 homogenate contained 13 mg of protein/ml and was stored at -70°C. S9 homogenate was used at a concentration of 10% (v/v)

in the assay.

BaP was used as the positive control.

Negative control plates for determination of the spontaneous reversion rate were treated with 400µl or less of 10% Tween 80, a non-toxic and non-

mutagenic dose.

No other experimental details are given in the paper, but reference is made

to the papers by Ames that describe the methodology.

Result : Addition of up to 400µl Tween 80 per plate did not alter the spontaneous

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reversion rates of either TA 98 or TA 100. Tween 80 had no effect on the mutagenicity of BaP, which induced similar numbers of revertants in TA 98 $\,$

with S9 activation whether dissolved in DMSO or Tween 80.

The crude oil sample was inactive in all bacterial mutagenicity tests.

Reliability : (4) not assignable

The numbers of revertants were not given. The result was simply stated by

the authors.

(43)

Type : Sister chromatid exchange assay

System of testing : Human lymphocytes

Year : 1982 GLP : no data

Test substance: Wilmington crude oil

Method: Heparinized blood was obtained from a healthy 25-year old male. [The

method for culture of the lymphocytes was not described but reference was

given which describes the method].

Benzo(a)pyrene (BaP) and N-methyl-N'-nitro-N-nitroso guanidine (MNNG)

were used as positive control substances.

The test sample was suspended in Tween 80 prior to testing. Each sample was weighed in a glass vial and then an equal weight of Tween 80 was added. The oil and detergent were stirred until thoroughly homogenized. Distilled water was then added dropwise with continuous stirring until a stable, homogenous suspension was obtained. The concentration of the mixture was then adjusted to 1% sample - 1% Tween 80 (w/v) by the addition of more distilled water. Lymphocyte cultures were treated 18 hours after initiation by

- (a) continuous exposure to test chemical without exogenous activation or
- (b) by exposure for 2.5 hours with activation by S9. [procedure described elsewhere by Stetka and Wolff (1976) for metabolic activation].

In tests with S9, cells were collected by centrifugation at 150 x g for 10 minutes, resuspended in 5 ml of McCoy's 5A medium containing only antibiotics and treated with microliter quantities of test material or solvent. An aliquot of 0.25 ml of S9 mix containing 10% S9 (v/v) was added to each culture. Cells were incubated for 2.5 hours with occasional mixing. The cells were collected, washed once in medium with 5% fetal bovine serum and resuspended in 8 ml of culture medium containing BrdUrd for further incubation. In experiments testing MNNG, lymphocytes that had been cultured for 18 hours were suspended in medium without serum. S9 or BrdUrd, treated for 2 hours with MNNG in acetone or acetone alone (controls), washed as above, resuspended in complete medium with BrdUrd and incubated further. At 68 hours after the cultures were initiated, Colcemid (0.01µg/ml) was added; 4 hours later, the cells were harvested by centrifugation, subjected to hypotonic shock for 7 minutes in 0.075M KCl, fixed in 3 changes of cold methanol-acetic acid (3:1) and spread on chilled, wet microslides that had been pre-cleaned in 95% ethanol. The preparations were stained by the Hoechst 33258-black light-Giemsa method and numbers of SCE were counted in 25 metaphase cells containing 40-48 chromosomes

Result

: Tween 80 was not cytotoxic and did not induce increases in the numbers of SCE in cultures of lymphocytes treated with 100 ppm Tween 80 for 54 hours without activation or for 2.5 hours with S9 activation. MNNG, a strong alkylating agent, induced a greater than 5-fold increase in

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SCE at a concentration of only 0.15 ppm.

The response to BaP in the lymphocyte cultures indicated that the S9 metabolic system was functional. Although increases in SCE of only 50% over the number in control cultures were induced, these increases are statistically significant (P<0.001). In the presence of S9 mix, only 1/16 the amount of BaP was required to induce the same number of SCE observed without S9, indicating that metabolism had occurred. However, 0.5 μ g/ml of BaP with S9 mix was toxic to these cultures, as indicated by poor cell morphology and a reduced number of cells in mitosis.

Wilmington crude did not induce an increase in SCE at any dose concentration with or without S9 activation.

The data are summarized in the following table.

Treatment	Dose	No. of	SCE/cell		
	(ppm)	cells	<u> ±S.E.</u>		
24 hours exposure, without S9					
Tween 80	0	_ 25	8.4±0.5		
	100	25	9.7±0.6		
B(a)P	0	25	7.7±0.6		
	4	25	10.8±1.2		
	8	25	15.1±0.9**		
Wilmington cr	ude oil				
Ü	40	25	10.7±1.2		
	50	25	9.9±0.8		
2.5 hours exp	osure, with S9				
Tween 80	40	25	9.6±0.0		
	100	25	8.0±0.5		
B(a)P	0	25	10.9±1.9		
	0.2	25	13.4±0.8**		
	0.5	10	15.9±1.2**		
	1.0	0	Toxic		
MNNG	0	25	8.7±0.4		
	0.015	23	11.7±0.5**		
	0.05	23	28.3±1.3**		
	0.15	21	48.5±2.2**		
Wilmington crude oil					
	20	25	8.8±0.7		
	30	25	6.6±0.5		
**	P<0.001				

Reliability

: (2) valid with restrictions

Complete description of study not given in publication. Some methods details published elsewhere

(43)

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5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay

Species : rat

Sex : male/female Strain : Sprague-Dawley

Route of admin. : dermal Exposure period : 13 weeks

Doses : 30, 125 and 500 mg/kg/day

Result : negative
Year : 1990
GLP : yes

Test substance : Lost Hills Light crude

Method : The animals used in this study were taken from a 13 week repeated dermal

administration study (Study 63834).

Bone marrow was removed from the femurs of five male and five female

rats from each dose group (0, 30, 125 and 500 mg/kg/day).

A nearly pure erythrocyte fraction was obtained and two slides prepared for each animal. 1000 PCEs (polychromatic erythrocyte) and 1000 NCEs (normochromatic erythrocyte) were scored to determine the percentage of micronucleated erythrocytes. 1000 erythrocytes were scored to determine

the ratio of PCEs to NCEs.

Result : The results are given in the following table. Standard deviations have not been included in the table. However, statistical analyses were conducted

and it was found that there were no significant differences between treated and control animals.

It was concluded that the test material was not cytotoxic to red blood cell formation and furthermore did not increase the formation of micronucleated PCEs or NCEs in the bone marrow.

Dose mg/kg	Sex	No of anima	PCE/NCE Is	MNPCEs (%) (%)	MNNCEs
0	F	5	1.34	0.1	0
0	M	5	0.79	0.02	0
0	M+F	10	1.06	0.06	0
30	F	5	1.26	0.04	0
30	M	5	0.68	0	0.02
30	M+F	10	0.97	0.02	0.01
125	F	5	1.18	0	0
125	M	5	1.17	0.06	0
125	M+F	10	1.17	0.03	0
500	F	5	1.25	0.02	0
500	M	5	0.83	0.04	0
500	M+F	10	1.04	0.03	0

PCE Polychromatic erythrocytes
NCE Normochromatic erythrocytes
%MNPCEs % Micronucleated PCEs

%MNNCEs % Micronucleated normochromatic erythrocytes

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Remark: Two further studies have been reported.

1. Belridge heavy crude.

The results of this study were the same as those for the study on Lost Hills Light described above. (Mobil study 63847)

2. Wilmington crude

Results in ICR Swiss mice showed that Wilmington crude oil had relatively little capacity to cause chromosome breakage and/or non-disjunction. (Lockard et al, 1982)

Reliability: (1) valid without restriction

(43) (64) (71) (72)

Type : Sister chromatid exchange assay

Species: mouseSex: maleStrain: ICRRoute of admin.: i.p.

Doses : 1.8, 3.6 &7.2 g/kg

Year : 1982 GLP : no data

Test substance: Wilmington crude

Method : The assay was carried out in groups of 3 male 10-14 week old Sch:ICR

mice.

The mice were lightly anesthetized with ether and a 50 mg tablet of BrdUrd was inserted beneath the skin on the dorsal side of the neck of each animal. After 1 hour, treatment was given by a single i.p. injection at doses of 1.8, 3.6 and 7.2 g/kg (equivalent to 0.8, 0.4 and 0.2 times the approximate LD50).

Control groups of mice were injected with B(a)P at doses of 0.16, 0.08 and 0.04 g/kg, Cyclophosphamide at doses of 0.01 and 0.005 g/kg in distilled water. Trioctanoin and distilled water were also given to negative solvent controls.

22 hours after treatment, animals were injected i.p. with Colchicin (10 mg/kg). 2 hours later the mice were sacrificed by cervical dislocation and their femurs were removed. The bone marrow cells were flushed out with saline. Pooled cells from the 2 femurs of each animal were treated with hypotonic KCl for 30 minutes, were fixed and were spread on slides. The slides were stained and the numbers of SCE counted in 25 metaphase cells from each animal, each metaphase having 36-42 chromosomes.

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Result : Control animals injected with the solvent trioctanoin had a mean of 5.3

SCE/cell. A slight but significant increase in SCE was found in animals treated with the highest dose only of Wilmington crude oil, but the response

was not dose related.

B(a)P and cyclophosphamide induced dose-related increases in SCE.

The actual results are shown in the following table.

Treatment	Dose	No. survivors/ No. treated	No. cells examined	SCE/ cell±S.E.
Trioctanoin	0.5 ml/ mouse	7/8	175	5.3±0.4
B(a)P	0.16 g/kg 0.08 0.04	3/3 3/3 3/3	75 75 75	9.7±0.6*** 8.5±0.5*** 7.8±0.5***
Cyclophospha		3/3	75	19.5±0.6***
Wilmington or	0.005	3/3	75 75	14.6±0.6***
Wilmington cru		0/0	75	0.0.0.4**
	7.2 g/kg	3/3	75	6.6±0.4**
	3.6	3/3	50	5.0 ± 0.3
	1.8	3/3	75	6.4±0.4
**	$D \cap C \cap C$			

** P<0.05 *** P<0.001

Reliability : (2) valid with restrictions

Probably not to GLP and method description is not comprehensive.

(43)

5.7 CARCINOGENICITY

Species: mouseSex: maleStrain: C3HRoute of admin.: dermalExposure period: 18 monthsFrequency of treatm.: Twice weekly

Doses : 50 mg per application Result : positive

Result : positive
Control group : yes
GLP : no data

Test substance: Two crudes C & D

Method: 50 mg undiluted test material was applied to the shorn interscapular region

of groups of 50 male mice. Application was twice weekly for 18 months or until grossly-observable cancer was found. Each animal was observed weekly for the appearance of tumors. The percentage of animals developing tumors and the time to appearance of first tumor were recorded. No distinction was made between histologically benign or

malignant lesions.

Result: The % tumors and average latencies for the two crudes C & D are shown

below.

	% Tumors*	Latency**
Crude C	33	76
Crude D	56	64

Based on Final Effective Number ** Average latency in weeks

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Test substance

: Two crudes were included in this study. They were characterized as follows:

	Crude C	Crude D
Sulfur (wt%)	0.21	2.54
Distillation yield (vol%)		
Int-120°F	0.0	3.3
120-350	2.5	18.3
350-550	41.9	19.5
550-700	21.0	12.2
700-1070	31.2	26.8
1070 btms	3.4	19.9
Composition of 550-700°F		
Fraction (wt%)	5.1	33.3
Monocycloparaffins	8.1	11.5
Polycycloparaffins	46.6	19.5
Total saturated hydrocarbons	59.8	64.3
Mononuclear aromatics	21.5	11.0
Di- and Trinuclear aromatics	14.4	21.8
Polynuclear aromatics	1.0	1.8
Total aromatic hydrocarbons	36.9	34.6
Resins	3.3	1.1
Asphaltenes	0.0	0.0
(2) valid with restrictions		

Reliability : (2) valid with restrictions

(42)

Species : mouse : male/female

Strain : C3H
Route of admin. : dermal
Exposure period : 105 weeks

Through the street in the street

Frequency of treatm. : Three times weekly

Doses : 25 mg per application

Control group : yes Year : 1988 GLP : no data

Test substance: Heavy crude oil, San Joaquin Valley

Method

: The study reported by Clarke et al was a comparison of the carcinogenicity of shale and petroleum crude oil and of several distillate streams derived from the two crude oils. Only the details relating to petroleum crude and the respective positive and negative controls are included in this summary. The crude oil, positive and negative control materials were applied three times weekly at a dose of 25 mg/application to 25 male and 25 female mice. The materials were applied to the shorn dorsal thoracic region which was shaved as necessary during the study. Dosing was continued for up to 105 weeks.

Animals were observed twice daily for overt signs of toxicity, moribundity and mortality. Animals were weighed weekly for the first 13 weeks and every two weeks thereafter. Animals were palpated weekly for external and internal masses.

Every animal was subjected to a complete necropsy. All organs and the remainder of the carcass were fixed and stored. Body weights and weights of liver, kidneys, brain and gonads were recorded. A microscopic examination was made of histological slides prepared from skin from a

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Result

control site, skin from treated site, kidney, liver, lungs, gonads, urinary bladder, spleen, sternal bone marrow and any other organ considered abnormal at necropsy.

Survival was affected by treatment with petroleum crude oil. After one year, survival was approximately 70% and by week 70 survival was approximately 50%. There were few survivors at 105 weeks. This is in contrast to the negative control group with 50% survival at the end of the study. Dermal irritation at the test site first appeared at 271 days and males developed irritation earlier than the females [no details given]. Necrosis occurred and was characterized as loss of integrity of the skin with visible cracking, separation and sloughing of skin often revealing underlying mesenchymal tissue.

There was no indication of toxic or oncogenic effects on internal organs.

The authors reported the occurrence of reactive hyperplasia in the spleen and bone marrrow and this was characterized by increased populations of lymphocytes, plasma cells and neutrophils or granulocyte precursors and was attributed to the inflammation, necrosis and other tissue alterations occurring at the test site. [There is no indication in the publication whether this finding occurred in the group treated with petroleum crude oil.]

The tumor incidences that were recorded are shown in the following table:

	Cruae oii	B(a)P	Control
Incidence (%)	37/44 (84)	49/49	0/46
Latency (weeks)	62±13	28±4	
Squamous cell carcinoma	29 (66)	49 (100)	0
Fibrosarcoma	7 (16)	0	0

Test substance

: The San Joaquin Valley crude oil is a heavy crude with the following characteristics:

Gravity (°API) 10.7 Boiling range (°F) 410-1071 Nitrogen (ppm wt) 8150 Sulfur (ppm wt) 10,510

Due to its high viscosity, the crude oil was administered as a 2:1 dilution in mineral oil.

Mineral oil USP was used as a negative control

B(a)P (0.15% w/v in mineral oil) was used as a positive control

: (2) valid with restrictions

Few experimental details are given in this publication. Despite this, the information may be used in the overall assessment of the carcinogenic potential of crude oil

(11)

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5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat Sex : female

Strain : Sprague-Dawley

Route of admin. : gavage Various Frequency of treatm. : 1987 **GLP** : no data

Test substance : Prudhoe Bay crude oil (Heavy crude oil)

Method : Presumed pregnant female Sprague-Dawley rats were used for this series

of studies.

Prudhoe Bay crude oil was administered by gavage according to the following schedules

As a single dose (5 ml/kg) on either day 3, 6, 11, 15 or 17 of 1. gestation.

2. Daily from gestation days 6-17 incl at a dose level of 1.0 or 2.0 ml/kg.

3. As a single dose on day six of gestation at either 2, 5, 7 or 10 ml/kg. Respective controls received equivalent amounts of saline.

On day 18 of pregnancy, animals were sacrificed and the numbers and position of implantations, resorptions and dead fetuses were recorded. The live fetuses were removed, weighed and inspected for gross external abnormalities with the aid of a dissection microscope. Skeletal examinations and examination of soft internal tissues were not carried out in this preliminary study.

Student's t-test was used for comparing control and experimental results. P values were derived from a 2-tailed table of Student's values for t. The level of significance chosen was P<0.05.

There were no maternal deaths following oral administration of PBCO although at high doses the animals exhibited signs of intoxication (crouching, indicative of acute irritation).

Maternal body weight changes were significantly less in animals that had been treated with PBCO at early days of gestation, in particular those animals given PBCO on day 6 of gestation only.

The body weight data are shown in the following tables. Note that although the author provided data on mean and standard deviation, for simplicity this summary only contains mean values (SDs are not shown). Statistically significant differences are indicated.

Body wt changes for dams given a single (5 ml/kg) dose of PBCO. Figures in parenthesis indicate group size

Treatment	Body v	weight o	change	(g on da	ays indicated
Day	0-6	6-11	11-15	15-18	<u>0-18</u>
Control (11)	30.4	32.6	28	30	121
3 (9)	32.75	28.25	23.5*	31	115.22
6 (10)	28.8	22.4*	24.2*	27.8	103.2*
11 (10)	34.8	35.6	16*	35.2	122.6
15 (10)	32.5	33.5	28	35.3	131.5
17 (10)	31.67	34	27.33	31.67	124.67

Significant from control (P<0.05)

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Result

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Effect of different doses of PBCO on dam body weight changes No. of animals shown in parenthesis

Treatment	Body v	veight c	hange (g on da	ys indicated)
	0-6	6-11	11-15	15-18	<u>0-18</u>
Control (8)	27.33	25.67	27.33	32	112.33
Dose (ml/kg) g	iven on	day 6 or	<u> 1ly</u>		
2 (8)	27.5	25	23*	27.5	1.3
5 (10)	26.21	18.08*	19.5*	26.92*	91.22*
7 (8)	26.67	17.33*	15.35*	26.92*	87.67*
10 (8)	30.67	14.33*	11*	20*	75.67*
Dose given on	days 6-	17 daily			
1 (10)	31	21*	23.5*	22*	97.5*
2 (10)	27.5	16.5	13*	17*	75*

^{*} Significant from control (P<0.05)

The author concluded that the reduced body weight gains were probably due to the high resorption rates that occurred.

Effects on fetal viability and development

The results of the effect of administering PBCO as a single dose of 5 ml/kg at different stages of gestation are summarized below:

Values shown are mean±SD

^{*} significant from control (p<0.05)

No. implants per dam	Resorptions/ dead fetuses (%)	No. live fetuses	Fetal weight (g)	Fetal crown-rump length (mm)
Day 0				
13.5±2.39	4.68±.93	13±1.24	1.267±.09	22.2±1.44
Day 3				
14.5±1.92	10.77±1.02*	11.25±1.5	1.19±.08*	20±1.73*
Day 6				
3.23±1.43	15.48±1.32*	11.02±1.49*	1.2±.09*	1.16±1.38
Day 11				
2.9±1.26	8.22±1.08*	11.4±.89*	1.287±.11	21.56±1.37
Day 15				
12.72±1.63	5.02±1.19	12.5±.71	1.313±.083	22.65±1.33
Day 17				
13±1.8	3.62±.89	12.33±1.15	1.304±.124	23.01±1.41

These results demonstrate that the incidence of resorptions and dead fetuses was increased in the animals given PBCO during the early days of pregnancy whereas those given PBCO during the later stages of pregnancy were unaffected. The greatest effect was on day 6 of gestation.

Repeated daily administration of low doses (1 or 2 ml/kg) on days 6 through 7 showed an additive effect and at a dose level of 2 ml/kg/day resulted in up to 40% fetal deaths. These data are shown in the following table.

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The effect of different doses of PBCO

Values shown are mean ± SD * significant from control (p<0.05)

No. implants per dam	Resorptions/ dead fetuses (%)	No. live fetuses	Fetal weight (g)	Fetal crown-rump length (mm)
Dose (ml/kg) 0 ml/kg 13.95±2.23	3.96±0.87	13.72±1.56	.309±.091	21.62±1.17
<u>Day 6</u> 2 ml/kg				
14±2.63 5 ml/kg	6.84±1.21	13.85±2.33	1.326±.119	21.78±1.54
13.26±1.95 7 ml/kg	12.67±1.18*	11.73±1.47*	1.216±.09*	21.54±1.27
12.93±2.35 10 ml/kg	13.23±1.35*	11.68±1.46*	1.2±.109*	21.28±.89
14.12±2.12	22.86±1.98*	10.5±1.59*	1.192±.108*	0.73±1.19
<u>Day 6-17 (dail</u> 1 ml/kg	y)			
13.92±2.46 2 ml/kg	17.5±2.02*	10±1.95*	1.184±.107	0.16±1.34*
12.93±1.98	43.8±1.92*	8.72±1.34*	1.182±.097*	.96±1.31*

In conclusion, administration of PBCO to pregnant females resulted in an increased incidence of resorptions, increased fetal death and decreased fetal body weight. These effects occurred at doses which were maternally

Reliability

(2) valid with restrictions

Probably not conducted to GLP. Study was a preliminary study and was limited in scope. Only information on embryo and fetotoxicity was derived in this study.

(40)

Species : rat Sex female

Strain Sprague-Dawley

Route of admin. : dermal Frequency of treatm. : Daily

Duration of test Days 0-19 of gestation **Doses** 30, 125 and 500 mg/kg/day **Control group** yes, concurrent no treatment

Year 1991 : no data **GLP**

Test substance : Belridge Heavy Crude

Method Groups of twelve presumed-pregnant Sprague-Dawley rats were

distributed into six groups as shown below. The test material was applied to the shorn dorsal skin once daily at the doses shown from day 0 to day 19 of gestation. The animals were shaved weekly and were fitted with

collars to prevent ingestion of the test material.

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Group	Dose level
Prenatal group	<u>S</u>
1	Sham control
2	30 mg/kg/day
3	125 mg/kg/day
4	500 mg/kg/day
Postnatal grou	<u>ps</u>
5	Sham control
6	500 mg/kg/day

Animals were observed at least once daily throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death. Dams and their litters were observed postpartum days 0 through 4. On day 0 postpartum, pups were examined for external malformations and variations and were observed daily for the presence of milk in their stomachs.

Body weights and food consumption of all prenatal group females were recorded at regular intervals throughout gestation and for the post natal groups throughout gestation and body weights only on postpartum days 0 and 4.

On day 20 of gestation all prenatal group animals were sacrificed. The abdominal cavity was exposed and the reproductive organs examined. Following removal of the uterus and ovaries the remains were subjected to macroscopic examination and the liver and thymus were weighed. The uterus and ovaries of each animal were examined grossly. The no. of corpora lutea per ovary was recorded. Ovaries of non pregnant females were examined and then discarded. The uterine contents of each pregnant animal were exposed and the number and location of all implantations were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation. Blood samples were collected at the time of sacrifice and these were used for a range of serum chemistry determinations. Each live fetus was weighed and its sex determined and examined for external anomalies. After gross examination the fetuses were equally distributed into two groups. One group was processed for an evaluation of visceral anomalies and the other group for skeletal anomalies. Females in the postnatal groups with surviving offspring were sacrificed on post partum day 4 and the abdominal cavity was exposed for a gross examination of the reproductive organs. The uterus was also examined and then discarded. All organs were examied macroscopically and the thymus and liver were weighed.

Remark Result

- : This study was also reported in the open literature by Feuston et al.
- : Clinical observations

Treatment related clinical observations in the prenatal and postnatal groups consisted of skin irritation in the 500 mg/kg/day group. This included erythema, edema, scabs and open sores at the treatment site. There was also a red vaginal discharge in animals in this group. One high dose prenatal female had excessive vaginal discharge and, after being found moribund, was sacrificed on gestation day 14. Uterine examination revealed that all but two fetuses had been resorbed.

Body weights and food intakes of the 500 mg/kg/day prenatal groups were reduced compared to controls (reduced by approx 31%). Other dose groups were unaffected.

Necropsy findings

With the exception of an approximately 11% increase in relative liver

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weight in the 500 mg/kg/day group, no other organ or relative organ weight changes were recorded. [The authors comment that the observed increase in relative liver weight was possibly attributable to the decreased body weight of this group].

A prominent vascular pattern of the liver was observed in one female of the 125 mg/kg/day group and two animals in the highest dose group.

Serum chemistry

The only observation was a 38% reduction of total bilirubin in the prenatal 500 mg/kg/day group.

Reproductive and fetal evaluations

Effects only occurred in the highest dose group (500 mg/kg/day). These included

an increase in the mean number/percent resorptions (5.3 compared to 1.3 and 35% compared to 7.8% respectively) and a corresponding decrease in litter size (10.8 compared to 14.5).

A decrease in mean fetal body weights for all viable fetuses (3.4 compared to 3.7).

Male fetuses in this dose group seemed to be more affected than females. Fetal skeletal anomalies recorded were limited to incomplete ossification of the nasal bones and caudal centra.

There were no treatment related visceral anomalies.

Post partum observations

During the post partum period there were no clinical observations of note except some persistent skin irritation. There were no findings at necropsy.

Litter data

Two females in the 500 mg/kg/day group had no viable offspring; their litters were totally resorbed. The incidence of pup mortality was two times greater in the group treated with crude oil during the lactation period. Mean duration of gestation was unaffected, but it was noted that the majority of treated females delivered either in the afternoon of day 22 or morning of day 23 whereas the controls delivered in the morning of day 22. [The biological significance of this observation is uncertain.]

Reliability : (1) valid without restriction

(29)(67)

Species : rat Sex : female

Strain : Sprague-Dawley

Route of admin. : dermal Frequency of treatm. : Daily

Duration of test : Days 0-19 of gestation

Doses : 125, 500 and 2000 mg/kg/day

Control group : yes, concurrent no treatment

NOAEL maternal tox. : 125 mg/kg bw NOAEL teratogen. : 500 mg/kg bw

Year : 1991 GLP : no data Test substance : Lost Hills Light

Method : Groups of twelve presumed-pregnant Sprague-Dawley rats were

distributed into six groups as shown below. The test material was applied

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to the shorn dorsal skin once daily at the doses shown from day 0 to day 19 of gestation. The animals were shaved weekly and were fitted with collars to prevent ingestion of the test material.

Group	Dose level
Prenatal group	<u>os</u>
1	Sham control
2	125 mg/kg/day
3	500 mg/kg/day
4	2000 mg/kg/day
Postnatal grou	<u>ips</u>
5	Sham control
6	20000 mg/kg/day

Animals were observed at least once daily throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death. Dams and their litters were observed postpartum days 0 through 4. On day 0 postpartum, pups were examined for external malformations and variations and were observed daily for the presence of milk in their stomachs.

Body weights and food consumption of all prenatal group females were recorded at regular intervals throughout gestation. For the post natal groups body weights and food intakes were recorded throughout gestation but only body weights were recorded on postpartum days 0 and 4. On day 20 of gestation all prenatal group animals were sacrificed. The abdominal cavity was exposed and the reproductive organs examined. Following removal of the uterus and ovaries the remains were subjected to macroscopic examination and the liver and thymus were weighed. The uterus and ovaries of each animal were examined grossly. The number of corpora lutea per ovary was recorded. Ovaries of non-pregnant females were examined and then discarded. The uterine contents of each pregnant animal were exposed and the number and location of all implantations were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation. Blood samples were collected at the time of sacrifice and these were used for a range of serum chemistry determinations.

Each live fetus was weighed and its sex determined and examined for external anomalies. After gross examination the fetuses were equally distributed into two groups. One group was processed for an evaluation of visceral anomalies and the other group for skeletal anomalies. Females in the postnatal groups with surviving offspring were sacrificed on post partum day 4 and the abdominal cavity was exposed for a gross examination of the reproductive organs. After examination, the uterus was discarded. All organs were examined macroscopically and the thymus and liver were weighed.

Remark Result This study was also reported in the open literature by Feuston et al. There were only few clinical observations that were considered to be treatment-related and these were confined to the high dose group (2000 mg/kg/day). The observations consisted of a red vaginal discharge, and one female in this group was pale in color. Slight skin irritation occurred in a few animals but it is not clear whether this was compound-related or self inflicted because the animals attempted to groom themselves, despite the fact that they had been fitted with collars. It is possible also that because of this some ingestion of test material may have occurred. Animals in the 500 and 2000 mg/kg/day groups gained less weight than the

Animals in the 500 and 2000 mg/kg/day groups gained less weight than the controls over the gestation period (80% and 60% of controls respectively).

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Food consumption for these two groups was also less than controls for the first 10 days of gestation but was similar to controls thereafter. At necropsy, it was noted that there were more animals in the 2000 mg/kg/day group with a small thymus. Absolute and relative thymus weights were reduced in this group by approximately 50%. A reduction in absolute thymus weight was also recorded for the 500 mg/kg/day group,

Liver weights (absolute and relative) were increased in the 500 and 2000 mg/kg group, but only the relative organ weights were statistically significant (11 and 18% increases for the 500 and 2000mg groups respectively).

Serum chemistry differences were only observed in the 500 and 2000 mg/kg/day groups These changes were as follows:

Parameter	Dose group (mg/kg/day)	
	500	2000
AST		+27%
ALT		+37%
Alkaline phosphatase		+64%
Cholesterol		+22%
Triglycerides	-42%	-62%
Total bilirubin	-35%	-49%
A/G ratio		+15%
Phosphorus		+20%
SDH		+67%

but the difference was not statistically significant.

All other serum chemistry measurements were unaffected by treatment.

Reproductive evaluations

The only effect was a significant increase in mean number/% resorptions (6-fold) and a corresponding decrease in litter size (38%) in the 200 mg/kg/day group.

Fetal evaluations

Mean fetal body weights were reduced by approximately 13% in the 200 mg/kg/day group.

There was an increase in incomplete ossification of various skeletal structures which included nasal bones and vertebrae but no increases in visceral abnormalities. The data are shown below:

Date are shown as fetal incidence/litter incidence

Anomoly	Dose group (mg/kg/day)			
	0	125	500	2000
Ossification vari	ants			
nasal bones	1.0/8.3	15b/55	33b/82b	96b/100b
Thoracic centra	4/33	5.5/36	8.5/55	15a/78
Caudal centra	1/8.3	9.9a/36	3.7/36	15b/67b
Sternebrae <2	2/8.3	6.6/36	7.3/36	23b/67a
Visceral malforn	<u>nations</u>			
Right-sided eso	phagus			
	0/0	-	0/0	4.1/22
a P<0.05	,			
b P<0.01				

In the prenatal group, the litter effects were;

Three females had no viable offspring, their litters were totally

Date: November 15, 2003

resorbed

Two females had their entire litters die by postpartum day 3

There was a significant increase in the number of stillborn pups (7

compared to 2 for controls)

Liveborn pups weighed less than the controls (-8% and -17% on

days 0 and 4 respectively).

: Lost Hills Light crude was shown to be maternally toxic at 500 and 2000 Conclusion

mg/kg/day and developmentally toxic at 2000 mg/kg/day.

Reliability : (1) valid without restriction

(29)(68)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type Study for sperm abnormality

In vitro/in vivo In vivo Species mouse Sex male

Strain : other: B6C3F1/Hap hybrid

Route of admin.

Frequency of treatm. Once daily for 5 days

Duration of test 40 days 1.0 & 2.1 g/kg Doses

Control group yes 1982 Year **GLP** no data

Test substance Wilmington crude

Method Groups of five 10-12 week old male mice were injected i.p. daily for 5 days

with test samples in trioctanoin at doses of 1 and 2.1 g/kg. Control mice were injected with trioctanoin (5x0.25 ml) or BaP (5x0.05 or 1x0.07g). After 35 days, the animals were sacrificed, the cauda epididymes were removed. combined and minced in 0.9% NaCl or Tyrode's solution and the sperm were stained by adding eosin Y solution. The preparations were spread on slides and air-dried. The percentage of abnormal sperm was determined in 500 sperm from each animal. For control of bias in scoring, positive and negative control slides from a previous experiment were included at a ratio

of 1 bias control slide to 5 new slides.

Result : The data from the study are given in the following table and show that

treatment with Wilmington crud did not cause an increase in the

percentage of abnormal sperm.

Sample	Dose	No. survivors/ no. treated	Abnormal sperm (%±SD)
Trioctanoin			
	5x0.25 ml	10/10	4.5±1.7
Wilmington c	rude		
	5x2.1 g/kg 5x1.0 g/kg	5/5 5/5	6.1±2.1 5.4±1.3
BaP	5x0.05 g/kg	10/10	19.7±16.6
	67 / 77		

(43)

ld: 8002-05-9 5. Toxicity

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5.9 SPECIFIC INVESTIGATIONS

Endpoint Mechanistic Studies Type Initiation/promotion assay

Species mouse Sex male

Charles River (ChR-CD) Strain

Route of admin. Dermal No. of animals 30 Vehicle undiluted Exposure period 180 day(s) See method Frequency of treatm. **Doses** See method

Control group yes 1981 Year GLP no data

South Louisiana crude Test substance

Method The authors summarized the protocol in the following table.

> Charles River (ChR-CD) male mice Six weeks old Animals:

> > Toe clipped for identification Backs shaved free of hair

Group size 30 mice per group

Housing 2 mice per cage

Diet Water and laboratory chow ad libitum

Observation Daily

Clipping of hair on the back Weekly, as needed

Treatments

Initiation: 50ul material as received Promotion: 2 weeks after initiation treatment 2.5µg phorbolmyristate acetate (PMA)/0.1ml acetone

Three times a week for 180 days

Parameters measured

Body weight, recorded weekly Time to development of first tumor

Tumors charted monthly

Mortality

Result Sample No. mice Av No. with tumors/

No. days to first tumors tumormouse with lasting bearing tumor >30 days mouse

Benzo[a]pyrene positive control

6.0 48 26

South Louisiana Crude

71 1.4

Toluene and PMA

	4	1.5	111
PMA alone	3	1.0	92
initiator. (4) not assignal The study was	ole a preliminary so	creen and was	not fully reported. The data
	initiator. (4) not assignal The study was	initiator. (4) not assignable The study was a preliminary so	

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201-14858B IUCLID

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C International Uniform ChemicaL Information Database
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C Column 6-80: Blockname / Fieldvalue
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С
B005 SUBST_MASTER_TAB
F001 8002-05-9
F002 Y26-001
```

F003 A complex combination of hydrocarbons. It consists predominantly of

- aliphatic, alicyclic and aromatic hydrocarbons. It may also contain
- small amounts of nitrogen, oxygen and sulfur compounds. This category
- encompasses light, medium, and heavy petroleums, as well as the oils
- extracted from tar sands. Hydrocarbonaceous materials requiring major
- chemical changes for their recovery or conversion to petroleum refinery
- feedstocks such as crude shale oils, upgraded shale oils and liquid coal
- fuels are not included in this definition.

EOB

С

B006 SUBST IDENT TAB

F001 8002-05-9

F002 Y28-001

F003 Y27-001

F004 8002-05-9

F005 1

EOR

F001 8002-05-9

F002 Y28-002

F003 Y27-006

F004 Petroleum

F005 2

EOR

F001 8002-05-9

F002 Y28-001

F003 Y27-002

F004 232-298-5

F005 3

EOR

F001 8002-05-9

F002 Y28-002

F003 Y27-030

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F004 Petroleum
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F002 Y28-002
F003 Y27-007
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F002 37
F001 8002-05-9
F009 N
F005 12031538
F006 22-02-2002
F007 12031538
F008 22-02-2002
F003 13-11-2003
F101 Robust summary of information on crude oil
F102 A35-01
EOB
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B004 COMPANY_TAB
F001 12031538
F003 American Petroleum Institute
F004 1220 L Street, NW
F005 Washington, DC
F006 20005
F008 A31-024
F010 202/682-8319
F012 202/682-8270
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С
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F007 13-11-2003
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F002 1
F003 1.1.1
F004 1
F005 1
F006 13-11-2003
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F007 06-10-2003

EOR

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F003 1.13

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F005 1

F006 09-10-2003

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F003 2.1

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F006 09-10-2003

F007 23-02-2003

EOR

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F003 2.14

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 2.2

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 2.4

F004 1 F005 1

F006 09-10-2003

F007 09-10-2003

EOR

F001 37

F002 1

F003 2.5

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 2.6.1

F004 1

F005 1

F006 09-10-2003

F007 30-09-2003

EOR

F001 37

F002 1

F003 3.1.1

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 3.3.2

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 3.6

F004 1

F005 1

F006 09-10-2003

F007 23-02-2003

EOR

F001 37

F002 1

F003 3.8

F004 2

F005 2

F006 13-11-2003

F007 06-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 1

F005 1

F006 09-10-2003

F007 17-03-2003

EOR

F001 37

F002 1

F003 4.1

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 4.1

F004 3

F005 3

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 4

F005 4

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 5

F005 5

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 6

F005 6

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 7

F005 7

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.1

F004 8

F005 8

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 4.2

F004 2

F005 2

F006 09-10-2003

F007 17-03-2003

EOR

F001 37

F002 1

F003 4.2

F004 3

F005 3

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F004 4

F005 4

F006 10-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F004 5

F005 5

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F004 6

F005 6

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F004 7

F005 7

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.2

F0048

F005 8

F006 09-10-2003

F007 06-10-2003

EOR

F001 37

F002 1

F003 4.3

F004 1

F005 1

F006 10-10-2003

F007 17-03-2003

EOR

F001 37

F002 1

F003 4.3

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 4.3

F004 3

F005 3

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.9

F004 1

F005 1

F006 10-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.9

F004 2

F005 2

F006 10-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.9

F004 3

F005 3

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.9

F004 4

F005 4

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 4.9

F004 5

F005 5

F006 09-10-2003

F007 01-10-2003

EOR

F001 37

F002 1

F003 5.1.1

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.1.1

F004 3

F005 3

F006 09-10-2003

F007 28-09-2003

EOR

F001 37

F002 1

F003 5.1.3

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.1.3

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.2.1

F004 1

F005 1

F006 03-11-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.2.1

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.2.1

F004 3

F005 3

F006 09-10-2003

F007 28-09-2003

EOR

F001 37

F002 1

F003 5.2.2

F004 1

F005 1

F006 09-10-2003

F007 22-05-2003

EOR

F001 37

F002 1

F003 5.2.2

F004 2

F005 2

F006 09-10-2003

F007 28-09-2003

EOR

F001 37

F002 1

F003 5.3

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.3

F004 2

F005 2

F006 09-10-2003

F007 28-09-2003

EOR

F001 37

F002 1

F003 5.4

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.4

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 3

F005 3

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 4

F005 4

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 5

F005 5

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.5

F004 6

F005 6

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.6

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.6

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.7

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.7

F004 2

F005 2

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.8.2

F004 1

F005 1

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.8.2

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F004 2
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F005 2

F006 09-10-2003

F007 13-10-2002

EOR

F001 37

F002 1

F003 5.8.2

F004 3

F005 3

F006 09-10-2003

F007 29-09-2003

EOR

F001 37

F002 1

F003 5.8.3

F004 1

F005 1

F006 09-10-2003

F007 12-02-2003

EOR

F001 37

F002 1

F003 5.9

F004 1

F005 1

F006 09-10-2003

F007 12-02-2003

EOB

С

B051 DS_COMPONENT_TAB

F001 37

F002 0

F003 8002-05-9

F012 N

F010 09-10-2003

F004 12031538

F005 22-02-2002

F006 12031538

F007 22-02-2002

F008 Robust summary of information on crude oil

F009 A35-01

EOR

F001 37

F002 1

F003 Crude oil

F012 Y

F010 09-10-2003

F004 12031538

F005 22-02-2002

F006 12031538

F007 22-02-2002

F008 Crude Oil

```
F009 A35-01
EOB
С
B101 GI_GENERAL_INFORM_TAB
F001 37
F002 1
F003 13-11-2003
F004 IUC4
F010 A04-06
F011 A19-03
EOB
С
B126 GI_ADD_REVIEWS_TAB
F001 37
F002 1
F003 06-10-2003
F004 IUC4
F007 IARC Review
EOB
С
B201 PC_MELTING_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F015 A36-002
F007 A02-03
F008 -30
F009 30
F010 A30-02
F011 A30-02
F012 P01-03: ASTM D97
F014 A03-02
EOB
С
B202 PC_BOILING_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F016 A36-002
F007 A02-03
F008 -1
F009 565
F010 1013
F011 P02-01
F012 A30-03
F013 P03-03: ASTM D86
F015 A03-02
EOB
```

B204 PC_VAPOUR_TAB

F001 37

```
F002 1
F003 09-10-2003
F004 IUC4
F015 A36-003
F014 A03-02
F018 A01-03: Crude oil
EOB
С
B205 PC_PARTITION_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F014 A36-002
F007 A02-03
F008 2
F009 6
F011 P07-04
EOB
С
B206 PC_WATER_SOL_TAB
F001 37
F002 1
F003 30-09-2003
F004 IUC4
F023 A36-003
F020 P09-03: Preparation of water soluble fraction
F021 1990
F022 A03-01
F025 A01-03: Crude oil
F030 C14-001
EOB
С
B212 PC_OTHER_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F008 A36-003
F009 CONCAWE
EOB
С
B301 EN_PHOTODEGRADATION_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F045 A36-003
F008 F01-01
F009 F02-05: according to Atkinson, 1990
F011 F03-01
F034 F06-03
```

F035 1000000

```
F036 F07-02
EOB
С
B302 EN_STABILITY_IN_WATER_TAB
F001 37
F002 1
F003 13-11-2003
F004 IUC4
F040 A36-002
F039 A03-01
EOB
С
B306 EN_DISTRIBUTION_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F010 A36-002
F007 F24-02
F008 F23-01
F009 1981
EOB
С
B309 EN_BOD_COD_TAB
F001 37
F002 1
F003 23-02-2003
F004 IUC4
F022 A36-003
F007 F32-03: Theoretical Oxygen Demand (TOD) was calculated
F008 1971
F009 A03-02
EOB
С
B311 EN_OTHER_TAB
F001 37
F002 2
F003 13-11-2003
F004 IUC4
F009 General
EOB
B401 EC_FISHTOX_TAB
F001 37
F002 1
F003 09-10-2003
F004 IUC4
F033 A36-002
F007 A01-03: CAS 8002-05-9 Crude oil - 0.5% paraffinic
F008 E01-04
F009 E02-0101
F010 E03-03
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F011 2001

```
F012 96
F013 E04-02
F014 E05-02
F031 A03-03
F032 A03-03
F050 C47-001
EOR
F001 37
F002 2
F003 09-10-2003
F004 IUC4
F033 A36-002
F007 A01-03: CAS 8002-05-9 Crude oil 3% paraffinic
F008 E01-04
F009 E02-0101
F010 E03-03
F011 2001
F012 96
F013 E04-02
F014 E05-02
F031 A03-03
F032 A03-03
F050 C47-001
EOR
F001 37
F002 3
F003 09-10-2003
F004 IUC4
F033 A36-004
F007 A01-03: Forties crude oil
F008 E01-05
F009 E02-0139
F010 E03-05: procedure as detailed in paper by Westlake (see Reference)
F014 E05-02
F021 A02-03
F022 258
F031 A03-01
F032 A03-03
F045 E35-02
EOR
F001 37
F002 4
F003 09-10-2003
F004 IUC4
F033 A36-004
F007 A01-03: Forties crude oil
F008 E01-05
F009 E02-0139
F010 E03-05: procedure as detailed in paper by Westlake (see Reference)
F011 1991
F012 96
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F013 E04-02 F014 E05-02

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F021 A02-03
F022 291
F031 A03-01
F032 A03-03
F045 E35-02
EOR
F001 37
F002 5
F003 09-10-2003
F004 IUC4
F033 A36-004
F007 A01-03: South Louisiana and Kuwait crude oils
F008 E01-05
F009 E02-0030
F010 E03-05: Procedure as detailed in paper by Anderson (see Reference)
F011 1974
F012 96
F013 E04-02
F014 E05-02
F021 A02-03
F022 2900
F023 80000
F031 A03-03
F032 A03-02
F045 E35-02
EOR
F001 37
F002 6
F003 09-10-2003
F004 IUC4
F033 A36-004
F007 A01-03: South Louisiana and Kuwait crude oils
F008 E01-05
F009 E02-0047
F010 E03-05: Procedure as detailed in paper by Anderson (see Reference)
F011 1974
F012 96
F013 E04-02
F014 E05-02
F021 A02-03
F022 6000
F023 14800
F032 A03-03
F045 E35-02
EOR
F001 37
F002 7
F003 09-10-2003
F004 IUC4
F033 A36-004
F007 A01-03: South Louisiana and Kuwait crude oils
F008 E01-05
```

F009 E02-0082

```
F010 E03-05: Procedure as detailed in paper by Anderson (see Reference)
F011 1974
F012 96
F013 E04-02
F014 E05-02
F021 A02-03
F022 3700
F023 9400
F031 A03-01
F032 A03-03
F045 E35-02
EOR
F001 37
F0028
F003 09-10-2003
F004 IUC4
F033 A36-003
F007 A01-03: Norman Wells crude oil
F008 E01-05
F009 E02-0139
F010 E03-05: Procedure as detailed in paper by Lockhart, Danell and Murray
* (see Reference)
F011 1987
F012 48
F013 E04-02
F014 E05-02
F031 A03-03
F032 A03-03
EOB
B402 EC_DAPHNIATOX_TAB
F001 37
F002 1
F003 29-09-2003
F004 IUC4
F032 A36-002
F007 A01-03: CAS 8002-05-9 Crude oil, 0.5% paraffinic
F008 E06-0034: Kelp Forest Mysid (Holmesymis costata)
F009 E07-04: USEPA 600 4-90-027
F010 2002
F011 96
F012 E04-02
F013 E05-02
F030 A03-03
F031 A03-03
F042 E01-04
F050 C47-001
EOR
F001 37
F002 2
F003 17-03-2003
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F004 IUC4

F032 A36-002 F007 A01-03: CAS 8002-05-9 Crude oil, 3% paraffinic F008 E06-0034: Kelp Forest Mysid (Holmesymis costata) F009 E07-04: USEPA 600 4-90-027 F010 2001 F011 96 F012 E04-02 F013 E05-02 F030 A03-03 F031 A03-03 F042 E01-04 F050 C47-001 EOR F001 37 F002 3 F003 09-10-2003 F004 IUC4 F032 A36-003 F007 A01-03: Samples of 8 crude oils F008 E06-0006 F009 E07-04: Procedure as detailed in paper by Franklin and Lloyd (see * Reference) F010 1982 F011 96 F012 E04-02 F013 E05-02 F026 LC50 F027 A02-03 F028 27 F029 110 F030 A03-01 F031 A03-02 F047 E35-02 EOR F001 37 F002 4 F003 10-10-2003 F004 IUC4 F032 A36-003 F007 A01-03: Samples of 8 crude oils F008 E06-0006 F009 E07-04: Procedure as detailed in paper by Franklin and Lloyd (see * Reference) F010 1982 F011 96 F012 E04-02 F013 E05-02 F026 LC50 F027 A02-03 F028 41

F029 119 F030 A03-01 F031 A03-02 F047 E35-02

EOR

F001 37

F002 5

F003 09-10-2003

F004 IUC4

F032 A36-004

F007 A01-03: Forties crude oil

F008 E06-0010

F009 E07-03

F010 1981

F011 24

F012 E04-02

F013 E05-02

F020 A02-03

F021 36

F030 A03-01

F031 A03-03

F045 E35-02

EOR

F001 37

F002 6

F003 09-10-2003

F004 IUC4

F032 A36-004

F007 A01-03: Forties crude oil

F008 E06-0010

F009 E07-03

F010 1981

F011 24

F012 E04-02

F013 E05-02

F020 A02-03

F021 42

F030 A03-01

F031 A03-03

F045 E35-02

EOR

F001 37

F002 7

F003 09-10-2003

F004 IUC4

F032 A36-002

F007 A01-03: Kuwait crude oil

F008 E06-0033: Holmesiysis costata

F009 E07-04: 40 CFR Part 797.1930, Mysid Shrimp Acute Test

F010 1993

F011 96

F012 E04-02

F013 E05-02

F026 LL50

F027 A02-03

F028 39.5

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F030 A03-03
F031 A03-03
F047 E35-02
EOR
F001 37
F0028
F003 09-10-2003
F004 IUC4
F032 A36-002
F007 A01-03: Kuwait crude oil
F008 E06-0023
F009 E07-04: 40 CFR Part 797.1930, Mysid Shrimp Acute Test
F010 1993
F011 96
F012 E04-02
F013 E05-02
F026 LL50
F027 A02-03
F028 618
F030 A03-03
F031 A03-03
F047 E35-02
EOB
С
B403 EC_ALGAETOX_TAB
F001 37
F002 1
F003 10-10-2003
F004 IUC4
F036 A36-002
F007 A01-03: CAS 8002-05-9 Crude oil - 0.5% paraffinic
F008 E08-0063: Macrocystis pyrifera
F010 2001
F012 48
F013 E04-02
F014 E05-02
F034 A03-03
F035 A03-03
F054 C47-001
EOR
F001 37
F002 2
F003 09-10-2003
F004 IUC4
F036 A36-002
F007 A01-03: CAS 8002-05-9 Crude oil 3% paraffinic
F008 E08-0063: Marine algae (Macrocystit pyrifera)
F009 E09-04: Chapman, G.A., D.L. Denton and J.M. Lazorchak. 1995
F010 2001
F012 48
F013 E04-02
F014 E05-02
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F034 A03-03

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F035 A03-03
F054 C47-001
EOR
F001 37
F002 3
F003 09-10-2003
F004 IUC4
F036 A36-002
F007 A01-03: Assam crude oil
F008 E08-0003
F009 E09-04: Procedure as detailed by Gaur and Singh (see Reference)
F010 1989
F011 E10-02
F012 15
F013 E04-01
F014 E05-02
F034 A03-03
F035 A03-02
EOB
С
B412 EC_OTHER_TAB
F001 37
F002 1
F003 10-10-2003
F004 IUC4
F008 A36-002
F009 Poorly water soluble mixtures
EOR
F001 37
F002 2
F003 10-10-2003
F004 IUC4
F008 A36-005
F009 Effect of crude oil on eggs
EOR
F001 37
F0023
F003 01-10-2003
F004 IUC4
F008 A36-005
F009 Effect of crude oil on Lobster larvae
EOR
F001 37
F002 4
F003 09-10-2003
F004 IUC4
F008 A36-005
F009 Effect of crude and refined oil on fresh and sea water organisms
EOR
F001 37
F002 5
F003 01-10-2003
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F004 IUC4

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F008 A36-005
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F009 Effect of crude oil on Sea urchin eggs

EOB

С

B501 TO_ACUTE_ORAL_TAB

F001 37

F002 1

F003 29-09-2003

F004 IUC4

F017 A36-003

F007 A01-03: Beryl crude (Light crude oil)

F008 T01-03

F009 T02-24

F011 1984

F012 A02-04

F014 5000

F015 T04-01

F016 A03-02

F019 T24-03

F020 5

F021 T52-003: None, undiluted

F022 T23-42

F023 Single dose: 5000 mg/kg

EOR F001 37

F002 3

F003 28-09-2003

F004 IUC4

F008 T01-03

EOB C

B503 TO_ACUTE_DERMAL_TAB

F001 37

F002 1

F003 29-09-2003

F004 IUC4

F017 A36-003

F007 A01-03: Beryl Crude (Light crude oil)

F008 T01-03

F009 T02-23

F011 1984

F012 A02-04

F014 2000

F015 T04-01

F016 A03-02

F019 T24-03

F020 3

F021 T52-003: None, undiluted

F022 T23-31

F023 Single dose: 2000 g/kg

EOR F001 37 F002 2

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F003 29-09-2003
F004 IUC4
F008 T01-03
EOB
С
B505 TO_SKIN_IRRITATION_TAB
F001 37
F002 1
F003 03-11-2003
F004 IUC4
F014 A36-002
F007 A01-03: Lost Hills Light Crude
F008 T02-23
F010 1985
F013 A03-02
F017 T49-001
F018 T50-001
F019 24
F020 T55-001
F021 6
F023 T52-003: Undiluted
EOR
F001 37
F002 2
F003 09-10-2003
F004 IUC4
F014 A36-003
```

F007 A01-03: Arab Light crude

F023 T52-003: Non, undiluted

F007 A01-03: Belridge heavy crude

F008 T02-23 F010 1984 F013 A03-02 F017 T49-001 F018 T50-001 F019 24 F020 T55-001 F021 6

EOR F001 37 F002 3

F003 28-09-2003 F004 IUC4 F014 A36-003

F008 T02-23 F010 1997 F011 T15-04 F012 T46-06 F017 T49-001 F018 T50-001 F019 4 F020 T55-001 F022 1.9

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EOB
С
B506 TO_EYE_IRRITATION_TAB
F001 37
F002 1
F003 22-05-2003
F004 IUC4
F014 A36-003
F007 A01-03: Beryl Crude (Light crude oil)
F008 T02-23
F010 1985
F017 T49-001
F018 .1
F019 T56-001
F022 6
F023 T51-002
EOR
F001 37
F002 2
F003 28-09-2003
F004 IUC4
F008 T02-23
EOB
С
B507 TO_SENSITIZATION_TAB
F001 37
F002 1
F003 29-09-2003
F004 IUC4
F015 A36-002
F007 A01-03: Lost Hills Light Crude
F008 T18-01
F009 T02-10
F011 1991
F013 T21-02
F014 A03-03
F017 20
F018 T53-001
F019 15
F020 T49-002
F021 T54-002
F022 T53-002
F023 15
F024 T49-002
F025 T54-002
F026 T53-002
F027 10
F028 T49-002
F029 T54-002
F030 T52-003: Mineral oil
EOR
```

F001 37 F002 2

```
F003 28-09-2003
F004 IUC4
F015 A36-003
F007 A01-03: Belridge heavy crude
F008 T18-01
F011 1991
F013 T21-02
F018 T53-001
F019 15
F020 T49-002
F022 T53-002
F023 10
F024 T49-002
F026 T53-002
F027 15
F028 T49-002
EOB
B508 TO_REPEATED_DOSE_TAB
F001 37
F002 1
F003 29-09-2003
F004 IUC4
F007 A01-03:Two crude oils
F008 T02-24
F009 T23-42
F010 T24-03
F011 T25-01
F013 1992
F015 5 days/week for 13 weeks
F017 30, 125 and 500 mg/kg/day
F018 T27-04
F029 A03-02
F032 C07-002
EOR
F001 37
F002 2
F003 29-09-2003
F004 IUC4
F030 A36-003
F007 A01-03: Prudhoe Bay Crude oil (Heavy crude oil)
F008 T02-18
F009 T23-10
F010 T24-03
F011 T25-03
F013 1990
F014 5 days
F015 Daily for five days
F017 2, 4, 8, 12 & 16 ml/kg/day
F018 T27-04
F029 A03-02
F032 C07-001
```

EOB

С

B509 TO_GENETIC_IN_VITRO_TAB

F001 37

F002 1

F003 09-10-2003

F004 IUC4

F016 A36-003

F017 1

F007 A01-03: Beryl Crude (Light crude oil)

F008 T30-19: Modified Ames Assay

F009 T31-18

F010 1984

F011 Ames test of a DMSO extract of test substance

F012 T32-02

F014 A03-02

F015 1 to 50µl/plate

EOR

F001 37

F002 2

F003 09-10-2003

F004 IUC4

F016 A36-002

F017 2

F007 A01-03: Lost Hills Light crude

F008 T30-06

F010 1991

F011 Chinese Hamster Ovary cells

F012 T32-02

F013 T33-02

F014 A03-02

F015 1 to 20 µl DMSO extract/ml culture medium

EOR

F001 37

F002 3

F003 09-10-2003

F004 IUC4

F016 A36-002

F0173

F007 A01-03: Belridge Heavy crude

F008 T30-06

F010 1991

F011 Chinese Hamster Ovary cells

F012 T32-02

F013 T33-02

F014 A03-02

F015 1 to 20 µl DMSO extract/ml culture medium

EOR

F001 37

F002 4

F003 29-09-2003

F004 IUC4

F016 A36-005

F017 4

```
F007 A01-03: South Louisiana crude (Light crude oil)
F008 T30-01
F010 1981
F012 T32-03
F014 A03-02
EOR
F001 37
F002 5
F003 09-10-2003
F004 IUC4
F016 A36-005
F017 5
F007 A01-03: Wilmington crude
F008 T30-01
F010 1982
F012 T32-03
F013 T33-02
F014 A03-02
EOR
F001 37
F002 6
F003 09-10-2003
F004 IUC4
F016 A36-003
F017 6
F007 A01-03: Wilmington crude oil
F008 T30-15
F010 1982
F011 Human lymphocytes
F014 A03-02
EOB
B510 TO_GENETIC_IN_VIVO_TAB
F001 37
F002 1
F003 29-09-2003
F004 IUC4
F018 A36-002
F007 A01-03: Lost Hills Light crude
F008 T34-07
F009 T02-24
F010 T23-42
F012 1990
F013 T24-03
F014 T25-01
F015 13 weeks
F016 30, 125 and 500 mg/kg/day
F017 A03-03
F020 T33-02
EOR
F001 37
```

F002 2

F003 09-10-2003

```
F004 IUC4
F018 A36-003
F007 A01-03: Wilmington crude
F008 T34-09
F009 T02-18
F010 T23-22
F012 1982
F013 T24-02
F014 T25-05
F016 1.8, 3.6 &7.2 g/kg
F017 A03-02
EOB
С
B511 TO_CARCINOGENICITY_TAB
F001 37
F002 1
F003 29-09-2003
F004 IUC4
F020 A36-003
F007 A01-03: Two crudes C & D
F008 T02-18
F009 T23-07
F010 T24-02
F011 T38-01
F014 18 months
F015 Twice weekly
F017 50 mg per application
F018 T27-07
F019 A03-02
F022 T33-03
EOR
F001 37
F002 2
F003 29-09-2003
F004 IUC4
F020 A36-003
F007 A01-03: Heavy crude oil, San Joaquin Valley
F008 T02-18
F009 T23-07
F010 T24-03
F011 T38-01
F013 1988
F014 105 weeks
F015 Three times weekly
F017 25 mg per application
F018 T27-07
F019 A03-02
EOB
B513 TO_DEVELOPMENTAL_TAB
F001 37
```

F002 1

F003 29-09-2003

```
F004 IUC4
F030 A36-002
F007 A01-03: Belridge Heavy Crude
F008 T02-24
F009 T23-42
F010 T24-01
F011 T25-01
F013 1991
F014 Days 0-19 of gestation
F016 Daily
F017 30, 125 and 500 mg/kg/day
F018 T27-04
F029 A03-02
EOR
F001 37
F002 2
F003 09-10-2003
F004 IUC4
F030 A36-002
F007 A01-03: Lost Hills Light
F008 T02-24
F009 T23-42
F010 T24-01
F011 T25-01
F013 1991
F014 Days 0-19 of gestation
F016 Daily
F017 125, 500 and 2000 mg/kg/day
F018 T27-04
F019 A02-03
F020 125
F022 T43-02
F023 A02-03
F024 500
F026 T43-02
F029 A03-02
EOR
F001 37
F0023
F003 09-10-2003
F004 IUC4
F030 A36-003
F007 A01-03: Prudhoe Bay crude oil (Heavy crude oil)
F008 T02-24
F009 T23-42
F010 T24-01
F011 T25-03
F013 1987
F016 Various
F029 A03-02
EOB
С
```

B018 TO_REPRODUCTION_OTHER_TAB

```
F001 37
F002 1
F003 12-02-2003
F004 IUC4
F007 C37-001: Study for sperm abnormality
F008 C08-002
F009 T02-18
F010 T23-48: B6C3F1/Hap hybrid
F011 T24-02
F012 T25-05
F014 Once daily for 5 days
F015 40 days
F016 A01-03: Wilmington crude
F017 1.0 & 2.1 g/kg
F018 T27-07
F020 1982
F021 A03-02
EOB
С
B019 TO_SPEC_INVEST_TAB
F001 37
F002 1
F003 09-10-2003
F004 IUC4
F005 A36-005
F007 C11-004
F010 C40-001: Initiation/promotion assay
F011 A01-03: South Louisiana crude
F012 T02-18
F013 T23-48: Charles River (ChR-CD)
F014 T24-02
F015 Dermal
F016 30
F017 T52-003: undiluted
F018 180
F019 C35-003
F020 See method
F021 See method
F024 1981
F025 A03-02
F027 C53-006
EOB
С
B601 TEXT_TAB
F002 37
F010 1.1.1
F004 1
F005 RM
F006 The CAS definition for Petroleum (Crude oil) is:
              "A complex combination of hydrocarbons. It consists
              predominantly of aliphatic, alicyclic and aromatic
```

hydrocarbons. It may also contain small amounts of

```
nitrogen, oxygen and sulfur comp
F007 The CAS definition for Petroleum (Crude oil) is:
**
                "A complex combination of hydrocarbons. It consists
                predominantly of aliphatic, alicyclic and aromatic
                hydrocarbons. It may also contain small amounts of
                nitrogen, oxygen and sulfur compounds. This
                                                                                                                 category
encompasses
   light, medium, and heavy
                                                                                petroleums, as well as the oils extracted
   from tar
                                sands".
                Hydrocarbonaceous materials requiring major
                                                                                                                 chemical
changes for
   their recovery or conversion
                                                                to petroleum refinery feedstocks such as
   crude
                                                shale oils, upgrade shale oils and liquid coal
fuels are not
   included in this definition.
   Crude oil contains hydrocarbons in the carbon number range
   from C1 to C60+. It also contains organometallic complexes,
   notably of sulfur and vanadium, and dissolved gases such as hydrogen
   Crude oils range from thin, light colored oils consisting
   mainly of gasoline-quality stock to heavy, thick tar-like
   materials.
   An "average crude oil has the following general composition:
                Carbon
                                                                84%
                Hydrogen
                                                14%
                Sulfur
                                                                1-3%
                                                1%
                Nitrogen
                Oxygen
                                                                1%
                Minerals and salts
                                                0.1%
   The chemical composition of crude oils can vary tremendously from
   different producing regions and even from within a particular formation.
   Examples of compositions of various whole crudes are shown
   in the following table
            Paraffins Naphthenes Aromatics Sulfur API
   Crude
   source
             % vol.
                      % vol.
                                % vol. % wt. gravity
                                    (°API)
   Light crudes
   Saudi light 63
                                              34
                        18
                                 19
                                        20
   South Louisiana
**
                           45
                                     19
                                            0
                                                 35
                   79
   Nigerian light
                   37
                                     9
                           54
                                           0.1
                                                 36
   North sea Brent
```

50

34

16

0.4

37

```
Beryl
           47
                    34
                                          37
   Lost Hills
                   Non-Aromatics 50%
                                           50
                                                  0.9
   Heavy crudes
   Prudhoe Bay 27
                                                36
                                                                   28
                                                                          0.9
                                                                                28
   Saudi Heavy 60
                         20
                                   15
                                          2.1
                                                28
   Venezuela Heavy
           35
                    53
                             12
                                    2.3
                                          24
   Belridge Heavy
           Non-aromatics 37%
                                   63
                                          1.1
**
   Mid-range crudes
   Kuwait
              63
                       20
                                24
                                       2.4
                                             31
   Venezuela Light
           52
                    34
                             14
                                    1.5
                                           30
   USA West Texas Sour
           46
                    32
                             22
                                    1.9
                                           32
**
   Crude oils may be categorized in either of several different ways e.g.
   Paraffinic vs naphthenic.
   Crude oils contain both paraffinic and naphthenic
   hydrocarbons but if there is a preponderance of parafffinic
   hydrocarbons present, the crude oil is referred to as a
   paraffinic crude. These crudes would be rich in straight and branched
   chain paraffins. Conversely a crude in which
   naphthenic hydrocarbons are predominant is referred to as a
   naphthenic crude. These crudes contain mainly naphthenic and aromatic
   hydrocarbons
   Sweet vs sour
   Crude oils may be referred to as either sweet or sour
   depending on the level of hydrogen sulfide present. A sweet crude has
   very little H2S whereas a sour crude has larger quantities of H2S present.
   Light vs heavy
   Crude oils may be divided into Light and Heavy crudes on the basis of
   their gravity.
   The API gravity is determined as:
                °API=
                                141.5/Specific gravity - 131.5
   Crude oils with gravity >33°API are considered as light
   crudes. Such crudes with a high percentage composition of
   hydrogen are usually more suitable for processing for
   gasoline production.
   Heavy crudes, ie those with gravity <28°API tend to contain
   more asphaltenes and are usually rich in aromatics. These
   heavy crudes require more steps in their processing.
```

**

** Information in this robust summary is presented for light and heavy crudes since this categorization distinguishes between crudes with a high paraffinic content (Light crudes) and those with a high aromatic/naphthenic content (Heavy crudes). This represents the extremes of the ranges of crudes available. F020 4489 **EOR** F002 37 F010 1.13 F004 1 F005 RE F006 International Agency for Cancer (1989) ** Occupational exposures in petroleum refining; crude oil and ** major petroleum fuels ** IARC monograph 45, IARC, Lyon, France F007 International Agency for Cancer (1989) ** Occupational exposures in petroleum refining; crude oil and ** major petroleum fuels ** IARC monograph 45, IARC, Lyon, France F020 4490 **EOR** F002 37 F010 1.13 F004 1 F005 RM F006 IARC reviewed the evidence for carcinogenicity of crude oil to man and animals and published the result in 1989. IARC concluded that: There is inadequate evidence for the carcinogenicity in humans of crude oil There is limited evidence F007 IARC reviewed the evidence for carcinogenicity of crude oil to man and animals and published the result in 1989. IARC concluded that: There is inadequate evidence for the carcinogenicity in humans of crude oil There is limited evidence for the carcinogenicity in experimental animals of crude oil The overall evaluation was Crude oil is not classifiable as to its carcinogenicity to humans * (Group 3) F020 4491 **EOR** F002 37 F010 2.1

F004 1 F005 RE

F006 American Society for Testing and Materials (ASTM), (1991)
** Annual book of ASTM standards, Section 5, Petroleum

- ** products, Lubricants and Fossil Fuels
- ** ASTM, Philadelphia, Pa

F007 American Society for Testing and Materials (ASTM), (1991)

- ** Annual book of ASTM standards, Section 5, Petroleum
- ** products, Lubricants and Fossil Fuels
- ** ASTM, Philadelphia, Pa

F020 4492

EOR

F002 37

F010 2.1

F004 1

F005 RE

F006 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F007 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F020 4493

EOR

F002 37

F010 2.1

F004 1

F005 RM

F006 The figures quoted are a typical range for the drop point as measured by

- * a standard oil industry procedure. For some low wax crudes, pour points
- * below -30 °C are obtained.

F007 The figures quoted are a typical range for the drop point as measured by

- * a standard oil industry procedure. For some low wax crudes, pour points
- * below -30 °C are obtained.

F020 4494

EOR

F002 37

F010 2.14

F004 1

F005 RE

F006 Banks, R.E. and King, P.J. (1986)

- ** Chemistry and Physics of Petroleum.
- ** In: Modern Petroleum Technology, Part I, 5th edition,
- ** Hobson, G.D. (Ed), John Wiley, Chichester.

F007 Banks, R.E. and King, P.J. (1986)

- ** Chemistry and Physics of Petroleum.
- ** In: Modern Petroleum Technology, Part I, 5th edition,
- ** Hobson, G.D. (Ed), John Wiley, Chichester.

F020 4495

EOR

F002 37

F010 2.14

F004 1

F005 RM

F006 Petroleum is a natural organic material consisting mainly of

- ** hydrocarbons. It occurs in both the gaseous and liquid
- ** states in geological traps. The liquid phase, after being
- ** freed from dissolved gas and any associated salt water, is
- ** known

F007 Petroleum is a natural organic material consisting mainly of

- ** hydrocarbons. It occurs in both the gaseous and liquid
- ** states in geological traps. The liquid phase, after being
- ** freed from dissolved gas and any associated salt water, is
- ** known as crude oil. All the information presented in this
- ** Data Set relates to crude oil.
- ** Crude oil is not a uniform substance since its physical and
- ** chemical properties vary from oilfield to oilfield and can
- ** even vary within wells at the same oilfield. At one
- ** extreme, crude oil is a light, mobile, straw-colored liquid
- ** containing a large proportion of hydrocarbons which are
- ** readily distilled at atmospheric pressure. At the other
- ** extreme, crude oil is a highly viscous, semi-solid, black
- ** substance from which little can be distilled at atmospheric
- ** pressure before thermal decomposition occurs.
- ** The most consistent property of crude oils is their
- ** relatively small range of elemental composition, as the
- ** ranges in the following table show:
- ** element composition (wt%)
- ** carbon 83.9 86.8
- ** hydrogen 11.0 14.0
- ** sulfur 0.06 8.0
- ** nitrogen 0.02 1.7
- ** oxygen 0.08 1.82
- ** metals 0.00 0.14
- **
- ** Crude oils are normally characterized in terms of three
- ** properties; density, viscosity and sulfur content. Crude
- ** oils are identified as either light (specific gravity
- ** <0.82), or medium (specific gravity 0.82 to 0.97), or heavy
- ** (specific gravity > 0.97). The viscosity is an expression
- of the mobility of the crude oil. The sulfur content has a
- ** marked influence on the refinery procedures to which the
- ** crude oil, and in particular its derivatives, will be
- ** subjected in order to produce acceptable products.
- ** Crude oils are also characterized in terms of their chemical composition,
- * specifically on the predominance of the hydrocarbon types that are
- * present. Modern practice tends to recognize two main types of crude,
- * namely paraffinic and naphthenic. Paraffinic crude oils are rich in
- * straight-chain and branched-chain alkanes, whereas in
- ** naphthenic crudes the main constituents are cycloparaffins
- ** and aromatic hydrocarbons. However, this is a simplified
- ** picture, as many crude oils fall between or outside these
- ** two types.

F020 4496

EOR

F002 37

F010 2.14

F004 1

F005 SO

F006 The technical information has been compiled by the Oil

- ** Companies' European Organization for Environmental Health
- ** Protection (CONCAWE), based at Madouplein-1, B-1210

- ** Brussel, Belgium, and this organization holds copies of the
- ** reference artic

F007 The technical information has been compiled by the Oil

- ** Companies' European Organization for Environmental Health
- ** Protection (CONCAWE), based at Madouplein-1, B-1210
- ** Brussel, Belgium, and this organization holds copies of the
- ** reference articles cited in this data set

F008 IUC4

F009 09-10-2003

F020 4497

EOR

F002 37

F010 2.2

F004 1

F005 RE

F006 American Society for Testing and Materials (ASTM), (1991)

- ** Annual book of ASTM standards, Section 5, Petroleum
- ** products, Lubricants and Fossil Fuels
- ** ASTM, Philadelphia, Pa

F007 American Society for Testing and Materials (ASTM), (1991)

- ** Annual book of ASTM standards, Section 5, Petroleum
- ** products, Lubricants and Fossil Fuels
- ** ASTM, Philadelphia, Pa

F020 4498

EOR

F002 37

F010 2.2

F004 1

F005 RE

F006 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F007 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F020 4499

EOR

F002 37

F010 2.2

F004 1

F005 RE

F006 Speight, J. G. (1990)

- ** Fuel science and technology handbook
- ** Marcel Dekker Inc., New York and Basel

F007 Speight, J. G. (1990)

- ** Fuel science and technology handbook
- ** Marcel Dekker Inc., New York and Basel

F020 4500

EOR

F002 37

F010 2.2

F004 1

F005 RM

F006 The method measures the distillation range of petroleum

** products. The figures quoted represent the approximate range

- ** for crude oils and are based on (a) the boiling point of
- ** n-butane for the lower value and (b) an upper limit quoted
- ** by Spei

F007 The method measures the distillation range of petroleum

- ** products. The figures quoted represent the approximate range
- ** for crude oils and are based on (a) the boiling point of
- ** n-butane for the lower value and (b) an upper limit quoted
- ** by Speight. In practice, atmospheric distillation of crude
- ** oil is not practiced above 275-300 °C, to avoid thermal
- ** decomposition of the residue. The residue is normally vacuum
- ** distilled in a subsequent operation.

F020 4501

EOR

F002 37

F010 2.4

F004 1

F005 RE

F006 Emergency Sciences Division (ESD) (1991)

** Experimental data, Environment Canada, Ottawa, ON.

F007 Emergency Sciences Division (ESD) (1991)

** Experimental data, Environment Canada, Ottawa, ON.

F008 IUC4

F009 13-11-2003

F020 4506

EOR

F002 37

F010 2.4

F004 1 F005 RE

F006 Environmental Emergencies Technology Division (EETD) (1984) Experimental

* data, Environment Canada, Ottawa, ON.

F007 Environmental Emergencies Technology Division (EETD) (1984) Experimental

* data, Environment Canada, Ottawa, ON.

F008 IUC4

F009 13-11-2003

F020 4508

EOR

F002 37

F010 2.4

F004 1

F005 RE

F006 Jokuty, P. S., Whiticar, Z., Fingas, M., Fieldhouse, B., Lambert, P. and

- * Mullin, J. (2002)
- ** Properties of crude oils and oil products.
- ** Environmental protection service, Environment Canada, Ottowa, Ontario.
- ** Internet version: 2002 via http://w

 $F007\ Jokuty,\ P.\ S.,\ Whiticar,\ Z.,\ Fingas,\ M.,\ Fieldhouse,\ B.,\ Lambert,\ P.\ and$

- * Mullin, J. (2002)
- ** Properties of crude oils and oil products.
- ** Environmental protection service, Environment Canada, Ottowa, Ontario.
- ** Internet version: 2002 via http://www.etcenter.org/spills

F020 4505

EOR

```
F002 37
F010 2.4
F004 1
F005 RE
F006 Oil and Gas Journal Databook (OGJ) (1999)
** Tulsa, OK,
F007 Oil and Gas Journal Databook (OGJ) (1999)
** Tulsa, OK.
F008 IUC4
F009 13-11-2003
F020 4507
EOR
F002 37
F010 2.4
F004 1
F005 RL
F006 Reliable with restrictions. Data obtained from a government (Environment
   Canada) database who sponsored the vapor pressure measurements cited by
   ESD and EETD.
F007 Reliable with restrictions. Data obtained from a government (Environment
   Canada) database who sponsored the vapor pressure measurements cited by
   ESD and EETD.
F020 4504
EOR
F002 37
F010 2.4
F004 1
F005 RM
F006 Vapor pressure measurements were provided for 10 petroleum crude oils
   originating from various locations throughout the world. The source of
   these data are from Environmet Canada Environmetal Technology Ceneter, a
   government-maintained da
F007 Vapor pressure measurements were provided for 10 petroleum crude oils
   originating from various locations throughout the world. The source of
   these data are from Environmet Canada Environmetal Technology Ceneter, a
   government-maintained database (Jokuty et al., 2002). Data cited by OGJ
   may be considered a secondary source, but the data cited by ESD and EETD
   were measured data sponsored by those agencies using a standardized
   method for measuring vapor pressure of petroleum products (1). As such,
   these 10 measurements provide a body of data adequate for use in the
   U.S. EPA HPV program and specific for the physical/chemical endpoint of
   vapor pressure.
** (1) ASTM D323, Standard Test Method for vapor Pressure of Petroleum
* Products (Reid Method)
F020 4503
EOR
F002 37
F010 2.4
F004 1
F005 RS
```

See the following table and Remarks

Not stated

F006 Vapor Pressure, kPa

** Temperature °C

section

Not stated Decomposition Vapor Pressures, kPa: Reid Vapor Product Pressure Name Origin Value, kPa Reference Alaska North Slope Alaska F007 Vapor Pressure, kPa See the following table and Remarks section Temperature °C Not stated Decomposition Not stated Vapor Pressures, kPa: Reid Vapor Product Pressure Value, kPa Name Origin Reference Alaska North Slope Alaska USA 19 **ESD 91** Arabian Medium Saudi Arabia 22.1 OGJ 99 Alif Yemen 45 OGJ 99 Amna Libya 27 OGJ 99 Ashtart Tunisia **OGJ** 99 13 Atkinson Beaufort Sea, Canada 6 **ESD 91** Alberta Sweet Mixed Blend Alberta, Canada 19 EETD 84 Abu Al Bu Khoush United Arab Emirates 24 OGJ 99 North Sea, UK Beryl 36 OGJ 99 Bombay High India 33 OGJ 99 F020 4502 **EOR** F002 37 F010 2.5 F004 1 F005 RE F006 CONCAWE (2000) ** ECB IUCLID dataset for crude oil F007 CONCAWE (2000) ** ECB IUCLID dataset for crude oil F020 4509 **EOR** F002 37 F010 2.5 F004 1 F005 RE F006 Log Pow Database (1993) ** Pomona College F007 Log Pow Database (1993)

** Pomona College

F020 4510 EOR F002 37

F010 2.5

F004 1

F005 RM

F006 The calculation was done by the CLOGP Version 3.5 program

- ** (calculation of Log partition coefficient octanol/water).
- ** The figures represent the spread of calculated and/or
- ** measured values for typical hydrocarbon components of crude
- ** oil. Calcu

F007 The calculation was done by the CLOGP Version 3.5 program

- ** (calculation of Log partition coefficient octanol/water).
- ** The figures represent the spread of calculated and/or
- ** measured values for typical hydrocarbon components of crude
- ** oil. Calculated values for higher molecular weight
- ** hydrocarbons will be above 6, but such values are notional,
- ** since no correlation has been established between calculated
- ** and experimental values.

F020 4511

EOR

F002 37

F010 2.6.1

F004 1

F005 CL

F006 Limited detail is provided for exact amounts of crude oil used for

- * preparing aqueous solutions, nor is there any information regarding the
- * composition of each of the crude oils tested, either as hydrocarbon type
- * or inorganic components (suc

F007 Limited detail is provided for exact amounts of crude oil used for

- * preparing aqueous solutions, nor is there any information regarding the
- * composition of each of the crude oils tested, either as hydrocarbon type
- * or inorganic components (such as sulfur). Also, no information on the GC
- calibration standard composition used to identify and quantify soluble
- * components in the equilibrated aqueous -oil solutions is provided.
- * Individual components of complex petroleum substances have specific and
- differing solubilities. At any particular loading rate, the resulting
- * aqueous concentration of each chemical constituent is a function of the
- * relative volume of the two phases (aqueous and the petroleum mixture),
- * the partition coefficient between the phases, the amount of component
- * present and the maximum water solubility of each component. Initially as
- * the petroleum mixture is added in amounts below the solubility limit of
- * the least soluble component the aqueous concentration increases
- proportionally until the least soluble component reaches a saturation
- * concentration, and only the more soluble components continue to dissolve,
- resulting in a two phase system. Further addition of the petroleum
- * mixture results in an aqueous concentration that is a non-linear function
- * of the amount added.

F020 4512

EOR

F002 37

F010 2.6.1

F004 1

F005 ME

F006 Individual saturated crude oil solutions were prepared by adding

- * approximately 10 ml of the respective oil to 50-100 ml of water in 125 ml
- * separatory funnels. Funnels were gently shaken for at least 48 hrs
- * either with a magnetic stir bar o

F007 Individual saturated crude oil solutions were prepared by adding

- * approximately 10 ml of the respective oil to 50-100 ml of water in 125 ml
- * separatory funnels. Funnels were gently shaken for at least 48 hrs
- * either with a magnetic stir bar or with a wrist action shaker, then
- * placed in a temperature bath for at least 48 hr prior to analysis.
- * Solubility at 5, 20 and 22 (±2C) °C was determined in both double
- * distilled water and salt water (3% NaCl). The effect of water to oil
- * ratio on the solubility of crude oil components was determined by
- * injecting oil into sealed vials completely filled with water, and mixed
- * at low speed for an equilibration period of 20 days. Analysis was
- * determined by purge-and-trap/GC; solid sorbent extraction with HPLC and
- * fluorescence analysis.

F020 4514

EOR

F002 37

F010 2.6.1

F004 1

F005 RE

F006 CONCAWE (1992)

- ** Ecotoxicological Testing Of Petroleum Products:
- ** Test Methodology.
- ** Report 92/56
- ** Brussels.

F007 CONCAWE (1992)

- ** Ecotoxicological Testing Of Petroleum Products:
- ** Test Methodology.
- ** Report 92/56
- ** Brussels.

F008 IUC4

F009 30-09-2003

F020 4516

EOR

F002 37

F010 2.6.1

F004 1

F005 RE

F006 CONCAWE (1996)

- ** Environmental risk assessment of petroleum substances:
- ** the hydrocarbon block method.
- ** Report 96/52
- ** Brussels.

F007 CONCAWE (1996)

- ** Environmental risk assessment of petroleum substances:
- ** the hydrocarbon block method.
- ** Report 96/52
- ** Brussels.

F020 4515

EOR

F002 37

F010 2.6.1

```
F004 1
F005 RE
```

F006 ECETOC (1996)

- ** Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable
- * Substances.
- ** Monograph 26.

F007 ECETOC (1996)

- ** Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable
- * Substances.
- ** Monograph 26.

F020 4517

EOR

F002 37

F010 2.6.1

F004 1

F005 RE

F006 ECETOC (1998)

- ** QSARS in the Assessment of the Environmental Fate and Effects of
- * Chemicals.
- ** Technical Report No. 74.

F007 ECETOC (1998)

- ** QSARS in the Assessment of the Environmental Fate and Effects of
- * Chemicals.
- ** Technical Report No. 74.

F020 4518

EOR

F002 37

F010 2.6.1

F004 1

F005 RE

F006 McAuliffe, C. (1966)

- ** Solubility in water of paraffin, cycloparaffin, olefin, acetylene,
- * cycloolefin and aromatic hydrocarbons.
- ** J. Phys. Chem. vol. 70, No 4, pp 1267-1275

F007 McAuliffe, C. (1966)

- ** Solubility in water of paraffin, cycloparaffin, olefin, acetylene,
- * cycloolefin and aromatic hydrocarbons.
- ** J. Phys. Chem. vol. 70, No 4, pp 1267-1275

F020 5445

EOR

F002 37

F010 2.6.1

F004 1

F005 RE

F006 Shiu, et al. Bridie, A.L. and Bos, J. (1990)

- ** The Water Solubility of Crude Oils and Petroleum Products. Oil & Chemical
- * Pollution 7, 1990, 57-84.

F007 Shiu, et al. Bridie, A.L. and Bos, J. (1990)

- ** The Water Solubility of Crude Oils and Petroleum Products. Oil & Chemical
- * Pollution 7, 1990, 57-84.

F008 IUC4

F009 13-11-2003

F020 4519

EOR

F002 37

F010 2.6.1

F004 1

F005 RS

F006 Fluorescence/HPLC analysis was inadequate in quantification of

- * hydrocarbon composition.
- ** Purge and trap/GC analysis results based on total benzene, toluene, ethyl
- * benzene+xylenes (combined concentration) and naphthalenes (BTE+XN as
- mg/L) a

F007 Fluorescence/HPLC analysis was inadequate in quantification of

- * hydrocarbon composition.
- ** Purge and trap/GC analysis results based on total benzene, toluene, ethyl
- * benzene+xylenes (combined concentration) and naphthalenes (BTE+XN as
- * mg/L) are reported along with viscosity (vis=c.p @ 20C) and density
- * (d=g/cm3 @ 20°C) for each oil at respective temperatures (°C) in
- distilled and saltwater (22° reported as ± 2°C)

**

Oil

BTE+XN (mg/l)

**	Oil		Distilled	Saltwater	
**			water		
**					
**	Norman Wells				
**	4.41 vis; 0.832 d				
**			30 (5)		25.5 (5)
**			29-33 (20)		
**			31.8-33.5 (2	22)	20 (22)
**					
**	4.7 vis; 0.84 d		33 (20)	14.8 (5)	
**					
**	Alberta: 4.98 vis; 0.835 d	25.02(22)			
**					
**	Swan Hills: (no vis. density)	35.1 (22)			
**					
**	Prudhoe Bay: 34.9 vis; 0.901 d	29.01 (22)			
**					
**	Lago Medio: 41.1 vis; 0.872 d	23.66-25.5 (22)		16.47 (22)	
**	Kopanoar: 17.5 vis; 0.9 d	10.42 (22)			
**	Murban: 4.37 vis; 0.824 d	28.62 (22)			
**	Hibernia B 33.2 vis; 0.837d			16.92 (22)	
**	Hibernia J (no vis., density)			7.75 (22)	

29.6 (22)

58 (22)

Distilled

Calturator

- ** Results for oil-water ratio testing were not quantified, but general
- * observations were stated as follows: concentrations of the water soluble
- * fractions decreases as the water-to oil ratio increases and the
- * composition of the water soluble fraction changes as the ratio changes.
- * At low water to oil ratios, the WSF is composed predominantly (80%) of
- * BTEX. As the water to oil ratio increases, these compounds account for a
- * smaller proportion of the dissolved compounds. At a water/oil ratio of
- * 10000, these compounds account for only 15-30% of the total WSF.

F020 4513

** Mobil A (no vis., density)

Mobil B (no vis., density)

EOR

F002 37

F010 3.1.1

F004 1

F005 RE

F006 Atkinson, R. (1990)

- ** Gas-phase tropospheric chemistry of organic compounds: a
- ** review
- ** Atmos. Environ. vol 24A, pp 1-41

F007 Atkinson, R. (1990)

- ** Gas-phase tropospheric chemistry of organic compounds: a
- ** review
- ** Atmos. Environ. vol 24A, pp 1-41

F020 4520

EOR

F002 37

F010 3.1.1

F004 1

F005 RE

F006 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F007 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F020 4521

EOR

F002 37

F010 3.1.1

F004 1

F005 RM

F006 Atkinson gives data which enables half lives to be

- ** calculated for the degradation of hydrocarbons in contact
- ** with hydroxyl radicals in the troposphere, under the
- ** influence of sunlight. Values for typical hydrocarbon
- ** constituents of crude o

F007 Atkinson gives data which enables half lives to be

- ** calculated for the degradation of hydrocarbons in contact
- ** with hydroxyl radicals in the troposphere, under the
- ** influence of sunlight. Values for typical hydrocarbon
- ** constituents of crude oils are as follows:

**	Constituent	Half-life
**		(days)
**	benzene	6.5
**	n-butane	3.2
**	n-hexane	1.4
**	toluene	1.3
**	cyclohexane	1.1
**	n-decane	0.69
**	n-tetradecane	0.42
**	naphthalene	0.37

- ** Hydrocarbons of carbon number greater than C20 will have
- ** little or no tendency to partition to air (see
- ** Sub-chapter3.2.2).

F020 4522

```
EOR
```

F002 37

F010 3.1.2

F004 1

F005 RE

F006 Harris, J. C. (1982)

- ** Rate of hydrolysis
- ** In: Handbook of chemical property estimation methods,
- ** Environmental behaviour of organic chemicals
- ** Chapter 7
- ** Lyman, W. J., Reehl, W. F. and Rosenblatt, D. H. (Eds)
- ** McGraw-Hill, New York

F007 Harris, J. C. (1982)

- ** Rate of hydrolysis
- ** In: Handbook of chemical property estimation methods,
- ** Environmental behaviour of organic chemicals
- ** Chapter 7
- ** Lyman, W. J., Reehl, W. F. and Rosenblatt, D. H. (Eds)
- ** McGraw-Hill, New York

F020 5443

EOR

F002 37

F010 3.1.2

F004 1

F005 RM

F006 Hydrolysis of an organic chemical is the transformation process in which

- * a water molecule or hydroxide ion reacts to form a new carbon-oxygen
- * bond. Chemicals that have a potential to hydrolyze include alkyl
- * halides, amides, carbamates, car

F007 Hydrolysis of an organic chemical is the transformation process in which

- * a water molecule or hydroxide ion reacts to form a new carbon-oxygen
- * bond. Chemicals that have a potential to hydrolyze include alkyl
- * halides, amides, carbamates, carboxylic acid esters and lactones,
- * epoxides, phosphate esters and sulfonic acid esters. The chemical
- components found in the materials that comprise the crude oil category
- * are hydrocarbons that are not subject to hydrolysis because they lack
- * functional groups that hydrolyze.

F020 5442

EOR

F002 37

F010 3.3.2

F004 1

F005 RE

F006 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F007 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F020 4523

EOR

F002 37

F010 3.3.2

F004 1

F005 RE

F006 Mackay, D., Patterson, S. and Shiu, W.Y. (1992)

- ** Generic models for evaluating the regional fate of chemicals
- ** Chemosphere Vol 24, pp 695-717

F007 Mackay, D., Patterson, S. and Shiu, W.Y. (1992)

- ** Generic models for evaluating the regional fate of chemicals
- ** Chemosphere Vol 24, pp 695-717

F020 4524

EOR

F002 37

F010 3.3.2

F004 1

F005 RM

F006 Distribution has been calculated according to Mackay Level I

- ** using the parameters defined in a paper by van der Zandt and
- ** van Leeuwen.
- ** The lower molecular weight components will mainly partition
- ** to air, with a maximum of about 1% of mono-ar

F007 Distribution has been calculated according to Mackay Level I

- ** using the parameters defined in a paper by van der Zandt and
- ** van Leeuwen.
- ** The lower molecular weight components will mainly partition
- ** to air, with a maximum of about 1% of mono-aromatic
- ** hydrocarbons partitioning to water. As the molecular
- ** weights increase, there is less tendency for the
- ** hydrocarbons to partition to air, the environmental
- ** distribution being preferentially to soil and sediment.
- ** Collectively, the wide molecular weight range of the
- ** hydrocarbons in crude oil will mean that at equilibrium,
- ** distribution will be mainly to air and soil, with much less
- ** than 1.0% being present in water.

F020 4525

EOR

F002 37

F010 3.3.2

F004 1

F005 RS

F006 Results for percent distribution of typical hydrocarbons

- ** found in crude oils are shown in tabular form as follows:
- ** air water soil sediment susp.matter biota
- ** propane
- ** 100.0 0.0 0.0 0.0 0.0

F007 Results for percent distribution of typical hydrocarbons

- ** found in crude oils are shown in tabular form as follows:
- * air water soil sediment susp.matter biota
- ** propane

**	100.0	0.0	0.0	0.0	0.0	0.0

- ** n-butane
- ** 100.0 0.0 0.0 0.0 0.0
- ** n-hexane
- ** 100.0 0.0 0.0 0.0 0.0 0.0
- ** n-octane
- ** 99.9 0.0 0.1 0.0 0.0 0.0
- ** n-decane

**	99.1	0.0	0.9	0.0	0.0	0.0	
**	n-tetradecane						
**	76.7	0.0	22.8	0.5	0.0	0.0	
**	n-eicosane						
**	0.0	0.0	97.7	2.2	0.1	0.0	
**	cyclohexane						
**	99.9	0.0	0.1	0.0	0.0	0.0	
**	benzene						
**	98.8	1.1	0.1	0.0	0.0	0.0	
**	toluene						
**	98.4	1.0	0.6	0.0	0.0	0.0	
**	p-xylene						
**	97.8	1.0	1.2	0.0	0.0	0.0	
**	o-xylene						
**	97.4	1.2	1.4	0.0	0.0	0.0	
**	ethylbenzene						
**	98.2	0.8	1.0	0.0	0.0	0.0	
**	n-butylbenzene						
**	91.0	0.4	8.5	0.2	0.0	0.0	
E020 4526							

F020 4526

EOR

F002 37

F010 3.6

F004 1

F005 ME

F006 Tests were run at 30 °C in fresh water using a respirometric

- ** procedure with crude oil at 50 and 70 mg/l. Prior to the
- ** test, the crude was "topped" at up to 100 °C to remove light
- ** ends, which comprised about 10% of the material

F007 Tests were run at 30 °C in fresh water using a respirometric

- ** procedure with crude oil at 50 and 70 mg/l. Prior to the
- ** test, the crude was "topped" at up to 100 $^{\circ}$ C to remove light
- ** ends, which comprised about 10% of the material

F020 4527

EOR

F002 37

F010 3.6

F004 1

F005 RE

F006 Bridie, A. L. and Bos, J. (1971)

- ** Biological degradation of mineral oil in sea water.
- ** J. Inst. Pet. Vol 57, pp 270-277

F007 Bridie, A. L. and Bos, J. (1971)

- ** Biological degradation of mineral oil in sea water.
- ** J. Inst. Pet. Vol 57, pp 270-277

F008 IUC4

F009 28-08-2003

F020 4528

EOR

F002 37

F010 3.6

F004 1

F005 RE

F006 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F007 CONCAWE (2000)

** ECB IUCLID dataset for crude oil

F020 4529

EOR

F002 37

F010 3.6

F004 1

F005 RS

F006 The BOD/TOD ratio after 10 days was 0.3 at 50 mg/l and 0.04

- ** at 70 mg/l.
- ** With the addition of nutrients, using ammonia at up to 4.6
- ** mg/l of nitrogen and phosphate at up to 15 mg/l of
- ** phosphorus, the BOD/TOD ratio increased to a maximum of 0.

F007 The BOD/TOD ratio after 10 days was 0.3 at 50 mg/l and 0.04

- ** at 70 mg/l.
- ** With the addition of nutrients, using ammonia at up to 4.6
- ** mg/l of nitrogen and phosphate at up to 15 mg/l of
- ** phosphorus, the BOD/TOD ratio increased to a maximum of 0.3
- ** after 5 days , and 0.34 after 10 days. In further studies,
- ** run at 10 °C in sea water in the presence of nutrients, the
- ** degradation rate was found to be lowered by a factor of 2 to
- ** 3 below that at 30 °C. The reference quotes the BOD5/TOD
- ** ratio, but does not give the individual values for BOD5 and
- ** TOD.

F020 4530

EOR

F002 37

F010 3.8

F004 2

F005 RE

F006 Atlas, R.M. and Bartha, R. (1973)

- ** Fate and effects of polluting petroleum in the marine
- ** environment.
- ** In: Residue Reviews of Pesticides and Other Contaminants in
- ** the TotalEnvironment, vol. 49, pp. 49-85

F007 Atlas, R.M. and Bartha, R. (1973)

- ** Fate and effects of polluting petroleum in the marine
- ** environment.
- ** In: Residue Reviews of Pesticides and Other Contaminants in
- ** the TotalEnvironment, vol. 49, pp. 49-85

F020 4533

EOR

F002 37

F010 3.8

F004 2

F005 RE

F006 Clark, R.B., (1989)

- ** Marine Pollution.
- ** 2nd Edition. Clarendon Press, Oxford, pp. 33-64

F007 Clark, R.B., (1989)

** Marine Pollution.

```
** 2nd Edition. Clarendon Press, Oxford, pp. 33-64
F020 4534
EOR
F002 37
F010 3.8
F004 2
F005 RE
F006 CONCAWE (2000)
** ECB IUCLID dataset for crude oil
F007 CONCAWE (2000)
** ECB IUCLID dataset for crude oil
F020 4535
EOR
F002 37
F010 3.8
F004 2
F005 RE
F006 CONCAWE (2001)
** Environmental classification of petroleum substances - summary data and
* rationale
** Report No. 01/54
** CONCAWE, Brussels
F007 CONCAWE (2001)
** Environmental classification of petroleum substances - summary data and
* rationale
** Report No. 01/54
** CONCAWE, Brussels
F020 5444
EOR
F002 37
F010 3.8
F004 2
F005 RE
F006 Zobell, C.E. (1963)
** The occurrence, effects and fate of oilpolluting the sea,
** Int. J. Air Wat. Poll., vol. 7, pp. 173-198
F007 Zobell, C.E. (1963)
** The occurrence, effects and fate of oilpolluting the sea,
** Int. J. Air Wat. Poll., vol. 7, pp. 173-198
F020 4536
EOR
F002 37
F010 3.8
F004 2
F005 RE
F006 Zobell, C.E. (1969)
** Microbial modification of crude oil in the sea.
** In: Joint Conference on Prevention and Control of Oil
** Proceedings of API-FWPCA Meeting, December15-17, New York.
** American Petroleum Institute publication, pp.
```

F007 Zobell, C.E. (1969)

** Microbial modification of crude oil in the sea.

- ** In: Joint Conference on Prevention and Control of Oil
- ** Spills.
- ** Proceedings of API-FWPCA Meeting, December15-17, New York.
- ** American Petroleum Institute publication, pp. 317ff

F020 4537

EOR

F002 37

F010 3.8

F004 2

F005 RM

F006 The world production of crude oil per year is of the order

- ** of 3 billion tonnes per year, of which about half is
- ** transported by sea. In his 1989 publication, Clark states
- ** that the best estimate of petroleum hydrocarbons entering the sea per

F007 The world production of crude oil per year is of the order

- ** of 3 billion tonnes per year, of which about half is
- ** transported by sea. In his 1989 publication, Clark states
- ** that the best estimate of petroleum hydrocarbons entering the sea per
- * year is about 3 million tonnes, of which about one million tonnes is
- * attributable to crude oil. Such pollution arises from the cleaning of
- * oil tanker compartments, offshore oil production operations, discharge of
- coastal refinery effluent and spillage from oil tankers. Crude oil also
- * enters the oceans by natural seepage from undersea locations.
- ** Particular attention has focused on major oil tanker
- ** spillages, notably involving the Torrey Canyon in 1969, the
- ** Amoco Cadiz in 1978 and the Exxon Valdez in 1990. As a
- ** result, the processes determining the fate of oil in
- ** seawater are reasonably well understood and have been
- ** reviewed by Atlas and Bartha.
- ** Initially, the oil spreads out as a film on the sea surface
- ** as a result of wind and wave action. The more volatile,
- ** lower molecular weight hydrocarbons are lost by evaporation.
- ** Polar compounds and the mono-aromatic hydrocarbons have an
- ** appreciable water solubility and are taken into solution. A key
- ancillary process is that of emulsification, since
- ** crude oil has a natural tendency to form emulsions in sea
- ** water. Such emulsions are usually of the oil-in-water type, but may also
- * be of the water-in-oil type. The latter are often of the intractable
- * 'chocolate mousse' type.
- ** Significant amounts of crude oil, particularly the higher
- ** molecular weight compounds, sink naturally, rolling along
- ** the ocean bottom picking up sand and shells and forming
- ** tarry balls which are resistant to degradation by any
- ** method. Hydrocarbons may also reach the bottom sediments by sorption
- * onto suspended particles which ultimately settle on the sea floor.
- ** Spilt oil also undergoes chemical changes, particularly
- ** oxidation by free radical mechanisms initiated by sunlight.
- ** The initial products of such reactions are hydroperoxides,
- ** and these in turn form compounds such as alcohols, acids and aldehydes,
- * many of which have an appreciable water
- ** solubility. Polymerization also occurs to yield intractable tarry
- * materials
- ** The bulk of spilt crude oil is biodegraded by the

- ** micro-organisms present in sea water. Emulsification to
- ** form oil-in-water emulsions yields small particles of crude
- ** oil that are biodegraded by bacteria, yeasts, fungi and
- ** actinomycetes. Many factors influence the rate of
- ** biodegradation, in particular temperature, dissolved oxygen
- ** concentration and the availability of nitrogen and
- ** phosphorus nutrients. Adapted micro-organisms are often
- ** found in ocean areas where crude oil spills are common.
- ** Zobell has calculated that where an adapted microbial
- ** population is available in well-aerated sea water at 20 to
- ** 30 °C, the rate of crude oil oxidation ranges from 0.02 to
- ** 0.2 g of oil oxidized/m²/day. The same author found
- ** experimentally that complete oxidation of 1.0 mg of
- ** hydrocarbon requires between 3 and 4 g of oxygen, i.e. it
- ** has a BOD of 3 to 4 mg oxygen/mg. Since the oxygen content
- ** of sea water is between 6 and 11 mg/liter, depending on
- ** salinity and temperature, this means that about 320 000
- ** liters of sea water is required to oxidize one liter of
- ** crude oil.
- ** Crude oil contains hydrocarbons of well-defined generic
- ** types that are biodegraded at different rates. n-Alkanes
- ** are readily degraded in sea water, since many
- ** micro-organisms can utilize them. Branched-chain or
- ** iso-alkanes are less readily biodegraded but they do
- ** ultimately biodegrade. The degradation of cycloalkanes has
- ** not been extensively studied, but the ring structure is
- ** resistant to biodegradation. Aromatic hydrocarbons are also resistant to
- * biodegradation, but a few micro-organisms are able to utilize them. High
- * molecular weight compounds, the tars and asphaltenes, degrade very slowly.

F020 4538

FOR

F002 37

F010 4.1

F004 1

F005 ME

F006 LL 50 at 96 hr calculated using the Trimmed Spearman-Karber

** Method (Hamilton, et al, 1977)

F007 LL 50 at 96 hr calculated using the Trimmed Spearman-Karber

** Method (Hamilton, et al, 1977)

F020 4539

EOR

F002 37

F010 4.1

F004 1

F005 RE

F006 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Fish acute toxicity test: Study No. 121258, test substance
- ** MRD-01-02. 2002.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F007 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Fish acute toxicity test: Study No. 121258, test substance
- ** MRD-01-02. 2002.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

```
F020 4540
EOR
F002 37
F010 4.1
F004 1
F005 RS
F006 Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5
** and 10, respectively for control, 1.4, 3.2, 8.5, 21 and 50
   mg/l treatments.
   96-hr LL50 = 21 mg/l, 95% C.I: 16-28 mg/l (as nominal
** loading rate)
   96-hour No Observed Effect Loadin
F007 Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5
** and 10, respectively for control, 1.4, 3.2, 8.5, 21 and 50
   mg/l treatments.
** 96-hr LL50 = 21 mg/l, 95% C.I: 16-28 mg/l (as nominal
** loading rate)
** 96-hour No Observed Effect Loading (NOEL) was 8.5 mg/l
   (observed.)
   Results are quoted in terms of 50% Lethal Loading (LL50),
   the loading rate of test substance resulting in 50%
   mortality of the test species exposed to the WAF.
        Measured BTEX (mg/l) in test treatment (mg/l load)
   Day
               Control
                               1.4
                                               3.2
                                                               8.5
                                                                               21
                                                                                               50
   0(new)
               ND
                               0.129
                                               0.382
                                                               0.835
                                                                               1.94
                                                                                               4.73
  1 (old) ND
               0.086 0.243 0.665 1.60 4.04
   3 (new) 0.005 0.148 0.301 0.973 1.86 NA
** 4 (old) 0.005 0.127 0.255 0.813 1.70 NA
** ND=not detected, NA=not analyzed due to 100% mortality
** The Practical Quantitation Limit (PQL) was approximately
** 0.0035 mg/l (3.5ng/ml) which corresponds to the
** concentration of the lowest standard analyzed.
F020 4541
EOR
F002 37
F010 4.1
F004 1
F005 TC
F006 Test solutions were prepared as water accommodated fractions
** (WAF). The control and dilution water was a reconstituted
** moderately hard water aerated prior to use. Water quality
** analyses for the dilution water was as follows: alkalinity =
** 72
F007 Test solutions were prepared as water accommodated fractions
** (WAF). The control and dilution water was a reconstituted
** moderately hard water aerated prior to use. Water quality
** analyses for the dilution water was as follows: alkalinity =
** 72 mg/l as CaCO3 , hardness =96 mg/l (as CaCO3), specific
** conductance = 320 µmhos, pH = 7.7 and dissolved oxygen = 8.6
   ppm.
```

Based on results of range-finding tests for a similar

petroleum product, measured loading rates of 1.4, 3.2, 8.5,

- ** 21 and 50 mg/l of crude oil to water were used to prepare
- ** test solutions for definitive toxicity tests. Test
- ** substance, added volumetrically, was mixed for each
- ** individual treatment in dilution water for 24 hours in
- ** 12-liter stoppered containers with approximately 20% vortex
- ** and less than 10% headspace volume. The WAFs were allowed to
- ** settle and cool to test temperature for 1-2 hours prior to
- ** drawing off the aqueous solutions for testing. Fish were 85
- ** days old at test initiation and were obtained from Pierce
- ** Associates Inc., West Buxton, ME, Lot 454. Loading of fish
- ** body mass to treatment was 0.551 g fish per liter of aqueous
- ** solution, mean length at termination was 4.1 cm (sd=0.2),
- ** and mean weight was 0.496 g (sd=0.078). Test vessels were
- ** 4-liter glass aspirator bottles with Teflon® covered
- ** neoprene stoppers. Two replicates per treatment and 5
- ** organisms per replicate were tested for each treatment and
- ** the control. Exposure containers were filled (no headspace
- ** volume) and tightly sealed to prevent volatilization. Test
- ** solution renewal was performed daily by removing at least
- ** 80% of the test solution and replacing it with fresh WAF
- ** solution prepared at least 24 hrs prior to use. Freshly
- ** prepared and old WAF test solutions were analyzed by GC-FID
- ** for concentrations of BTEX. Mean water temperature range was
- ** 13.8 (sd=0.1) °C. Test photoperiod was 16 hrs. light and 8
- ** hr dark, dissolved oxygen measurements ranged from 6.5 to
- ** 8.8 ppm, pH values between 6.8 and 7.8.

F020 4542

EOR

F002 37

F010 4.1

F004 2

F005 ME

F006 LL 50 at 96 hr calculated using the Trimmed Spearman-Karber

** Method (Hamilton, et al, 1977)

F007 LL 50 at 96 hr calculated using the Trimmed Spearman-Karber

** Method (Hamilton, et al, 1977)

F020 4543

EOR

F002 37

F010 4.1

F004 2

F005 RE

F006 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Fish acute toxicity test: Study No. 101158, test substance
- ** MRD-01-01.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F007 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Fish acute toxicity test: Study No. 101158, test substance
- ** MRD-01-01.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F020 4544

EOR

F002 37

```
F010 4.1
F004 2
F005 RS
F006 Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5
** and 10, respectively for control, 2.7, 6.8, 16, 40 and 109
   mg/l treatments.
** 96-hr LL50 = 41 mg/l, 95% C.I: 30-55 mg/l (as nominal
   loading rate)
** 96-hour No Observed Effect Loadin
F007 Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 0, 5
** and 10, respectively for control, 2.7, 6.8, 16, 40 and 109
** mg/l treatments.
   96-hr LL50 = 41 mg/l, 95% C.I: 30-55 mg/l (as nominal
** loading rate)
   96-hour No Observed Effect Loading (NOEL) was 16 mg/l
** (observed.)
   Results are quoted in terms of 50% Lethal Loading (LL50),
   the loading rate of test substance resulting in 50%
   mortality of the test species exposed to the WAF.
             Measured BTEX (mg/l) in test treatment
   (mg/l load)
   Day Control
                                              6.8
                                                              16
                                                                              40
                                                                                              109
                               2.7
** 0 (new) ND
                 0.093 0.366 0.541 1.36 2.02
  1 (old) 0.050 0.066 0.184 0.411 0.857 1.84
   3 (new) ND
                  0.096 0.272 0.585 1.28 2.01
  4 (old) 0.031 0.084 0.221 0.484 1.03 NA
** ND=not detected, NA=not analyzed due to 100% mortality
** Guideline/protocol deviations: DO level was 53%, not 60% of
** saturation.
F020 4545
EOR
F002 37
F010 4.1
F004 2
F005 TC
F006 Test solutions were prepared as water accommodated fractions
** (WAF). The control and dilution water was a reconstituted
   moderately hard water aerated prior to use. Water quality
   analyses for the dilution water was as follows: alkalinity =
** 78
```

F007 Test solutions were prepared as water accommodated fractions

- (WAF). The control and dilution water was a reconstituted
- ** moderately hard water aerated prior to use. Water quality
- ** analyses for the dilution water was as follows: alkalinity =
- ** 78 mg/l as CaCO3, hardness =105 mg/l (as CaCO3), specific
- conductance = 324 µmhos, pH = 7.6 and dissolved oxygen = 8.4

- ** Nominal loading rates of 0, 3.8, 16 and 74 mg/l were used
- ** to prepare test solutions for range-finding toxicity tests.
- ** Based on results of range-finding tests, measured loading
- rates of 0, 2.7, 6.8, 16, 40 and 109 mg/l of crude oil to
- ** water were used to prepare test solutions for definitive

- ** toxicity tests. Test substance, added volumetrically, was
- ** mixed for each individual treatment in dilution water for 24
- ** hours in 13-liter stoppered containers with approximately
- ** 20% vortex and less than 10% headspace volume. The WAFs were
- ** allowed to settle and cool to test temperature for 1-2 hours
- ** prior to drawing off the aqueous solutions for testing. Fish
- ** were 85 days old at test initiation and were obtained from
- ** Pierce Associates Inc., West Buxton, ME, Lot 454. Loading of
- ** fish body mass to treatment was 0.4 g fish per liter of
- ** aqueous solution, mean length at termination was 3.9 cm
- ** (sd=0.2), and mean weight was 0.466 g (sd=0.034). Test
- ** vessels were 4-liter glass aspirator bottles with Teflon®
- ** covered neoprene stoppers. Two replicates per treatment and
- ** 5 organisms per replicate were tested for each treatment and
- ** the control. Exposure containers were filled (no headspace
- ** volume) and tightly sealed to prevent volatilization. Test
- ** solution renewal was performed daily by removing at least
- ** 80% of the test solution and replacing it with fresh WAF
- ** solution prepared at least 24 hrs prior to use. Freshly
- ** prepared and old WAF test solutions were analyzed by GC-FID
- ** for concentrations of BTEX. Water temperature range was 14.3
- ** to 14.9 °C. Test photoperiod was 16 hrs. light and 8 hr
- ** dark, dissolved oxygen measurements ranged from 5.4 to 8.8
- ** ppm, pH values between 6.8 and 7.8.

F020 4546

EOR

F002 37

F010 4.1

F004 3

F005 ME

F006 The test species was the Rainbow Trout.

- ** Crude oil was added to water at 8 concentrations in the range 57 to 1330
- * mg/l in open vessels fitted with shielded propeller stirrers. A constant
- * rate of stirring was maintained during the 7-day expo

F007 The test species was the Rainbow Trout.

- ** Crude oil was added to water at 8 concentrations in the range 57 to 1330
- * mg/l in open vessels fitted with shielded propeller stirrers. A constant
- * rate of stirring was maintained during the 7-day exposure period to the
- * fish.

F020 4547

EOR

F002 37

F010 4.1

F004 3

F005 RE

F006 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)
- * Conference on Environmental Sciences and Sustainable Development,
- * University of Sh

F007 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)

- * Conference on Environmental Sciences and Sustainable Development,
- University of Sheffield, April 1991.

F020 4549

EOR

F002 37

F010 4.1

F004 3

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to be lower than those cited. These data are
- * also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by API crude oil task group as '3', not reliable.

F020 4550

EOR

F002 37

F010 4.1

F0043

F005 RS

F006 The results were as follows:

**

** 24-hour LC50 = 557 mg/l

** 96-hour LC50 = 258 mg/l

* 168-hour LC50 = 253 mg/l

**

- ** As the tests were run in open vessels, significant
- ** evaporative losses of lower hydrocarbons will have occurred
- ** and h

F007 The results were as follows:

**

- ** 24-hour LC50 = 557 mg/l
- ** 96-hour LC50 = 258 mg/l
- ** 168-hour LC50 = 253 mg/l

**

- ** As the tests were run in open vessels, significant
- ** evaporative losses of lower hydrocarbons will have occurred
- ** and hence the measured LC50 values underestimate the true
- ** ecotoxicity.

F020 4548

EOR

F002 37

F010 4.1

F0043

F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

F008 IUC4

F009 09-10-2003

F020 4571 **EOR** F002 37 F010 4.1 F004 4 F005 ME F006 The test species was the Rainbow Trout. Dispersions of oil in water at 8 concentrations in the range 57 to 1330 mg/l were prepared by shaking the constituents together in closed jars for 5 minutes using a reciprocating table. Fish tests we F007 The test species was the Rainbow Trout. Dispersions of oil in water at 8 concentrations in the range 57 to 1330 mg/l were prepared by shaking the constituents together in closed jars for 5 minutes using a reciprocating table. Fish tests were run in open vessels. F020 4551 **EOR** F002 37 F010 4.1 F004 4 F005 RE F006 Westlake, G.E.(1991 ** Aquatic test media preparation for low solubility materials, Proceedings of the Society of Environmental Toxicology and Chemistry (SETAC) Conference on Environmental Sciences and Sustainable Development, University of Sh F007 Westlake, G.E.(1991 ** Aquatic test media preparation for low solubility materials, Proceedings of the Society of Environmental Toxicology and Chemistry (SETAC) Conference on Environmental Sciences and Sustainable Development, University of Sheffield, April 1991. F020 4554 **EOR** F002 37 F010 4.1 F004 4 F005 RM F006 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC50 values are expected to F007 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the LC50 values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '3', not reliable.

F020 4553

EOR

F002 37

F010 4.1

F004 4

F005 RS

F006 The results were as follows:

```
**
       24-hour LC50 = 756 mg/l
       96-hour LC50 = 291 mg/l
       168-hour LC50 = 133 mg/l
   As the tests were run in open vessels, significant
   evaporative losses of lower hydrocarbons will have occurred
** and he
F007 The results were as follows:
       24-hour LC50 = 756 mg/l
       96-hour LC50 = 291 mg/l
       168-hour LC50 = 133 mg/l
** As the tests were run in open vessels, significant
** evaporative losses of lower hydrocarbons will have occurred
   and hence the measured LC50 values underestimate the true
** ecotoxicity.
F020 4552
EOR
F002 37
F010 4.1
F004 4
F005 SO
F006 CONCAWE Bruxelles
F007 CONCAWE Bruxelles
F008 IUC4
F009 09-10-2003
F020 4572
EOR
F002 37
F010 4.1
F004 5
F005 ME
F006 The test species was the Sheepshead Minnow. Dispersions of two crude oil
   samples in water were prepared by shaking the constituents together
   vigorously for 5 minutes on a shaker platform. Fish tests were run at 5
   concentrations. Figures q
F007 The test species was the Sheepshead Minnow. Dispersions of two crude oil
   samples in water were prepared by shaking the constituents together
   vigorously for 5 minutes on a shaker platform. Fish tests were run at 5
** concentrations. Figures quoted are for the loading rates.
F020 4555
EOR
F002 37
F010 4.1
F004 5
```

F006 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.
- * Biol.,vol.27, pp. 75-88.

F005 RE

F007 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.

```
* Biol.,vol.27, pp. 75-88.
F008 IUC4
F009 13-11-2003
F020 4556
EOR
F002 37
F010 4.1
F004 5
F005 RM
F006 These data are included to provide supporting evidence of the expected
   cumulative toxicity of the soluble components in crude oil. Since
   significant evaporative losses of lower hydrocarbons may have occurred,
   the LC50 values are expected to
F007 These data are included to provide supporting evidence of the expected
   cumulative toxicity of the soluble components in crude oil. Since
   significant evaporative losses of lower hydrocarbons may have occurred,
   the LC50 values are expected to be lower than those cited. These data are
   also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
   These data are ranked by API crude oil task group as '3', not reliable.
F020 4558
EOR
F002 37
F010 4.1
F004 5
F005 RS
F006 The results were as follows:
    For Kuwait crude:
       48-hour LC50 = 80\ 000+ mg/l
       96-hour LC50 = 80 000+ mg/l
    For South Louisiana crude:
       48-hour LC50 = 33 000 mg/l
       96-hour LC50 = 29 000 mg/l
   Parallel tests run with
F007 The results were as follows:
    For Kuwait crude:
       48-hour LC50 = 80\ 000+ mg/l
       96-hour LC50 = 80\ 000+ ma/l
    For South Louisiana crude:
       48-hour LC50 = 33 000 mg/l
       96-hour LC50 = 29 000 mg/l
   Parallel tests run with water-soluble fractions (WSF) failed to produce
   any meaningful results.
F020 4557
EOR
F002 37
F010 4.1
F004 6
F005 ME
```

F006 The test species was the Longnose Killifish. Dispersions of two crude oil

- * samples in water were prepared by shaking the constituents together
- vigorously for 5 minutes on a shaker platform. Fish tests were run at 5

* concentrations. Figures

F007 The test species was the Longnose Killifish. Dispersions of two crude oil

- * samples in water were prepared by shaking the constituents together
- * vigorously for 5 minutes on a shaker platform. Fish tests were run at 5
- * concentrations. Figures quoted are for the loading rates.

F020 4560

EOR

F002 37

F010 4.1

F004 6

F005 RE

F006 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.
- * Biol.,vol.27, pp. 75-88.

F007 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.
- * Biol.,vol.27, pp. 75-88.

F008 IUC4

F009 13-11-2003

F020 4562

EOR

F002 37

F010 4.1

F004 6

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to be lower than those cited. These data are
- * also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by API crude oil task group as '3', not reliable.

F020 4561

EOR

F002 37

F010 4.1

F004 6

F005 RS

F006 The results were as follows:

- ** For Kuwait crude:
- * 48-hour LC50 = 14 800 mg/l
- ** 96-hour LC50 = 14 800 mg/l
- ** For South Louisiana crude:
- ** 48-hour LC50 = 6000 mg/l
- ** 96-hour LC50 = 6000 mg/l

F007 The results were as follows:

- ** For Kuwait crude:
- ** 48-hour LC50 = 14 800 mg/l

```
** 96-hour LC50 = 14 800 mg/l

** For South Louisiana crude:

** 48-hour LC50 = 6000 mg/l

** 96-hour LC50 = 6000 mg/l

F020 4559
```

EOR

F002 37

F010 4.1

F0047

F005 ME

F006 The test species was the Tidewater Silverside. Dispersions of two crude

- * oil samples in water were prepared by shaking the constituents together
- * vigorously for 5 minutes on a shaker platform. Fish tests were run at 5
- ** concentrations. Figure

F007 The test species was the Tidewater Silverside. Dispersions of two crude

- * oil samples in water were prepared by shaking the constituents together
- * vigorously for 5 minutes on a shaker platform. Fish tests were run at 5
- ** concentrations. Figures quoted are for the loading rates.

F020 4563

EOR

F002 37

F010 4.1

F0047

F005 RE

F006 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.
- * Biol.,vol.27, pp. 75-88.

F007 Anderson, J.W. et al.(1974)

- ** Characteristics of dispersions and water-soluble extracts of crude and
- * refined oils, and their toxicity to estuarine crustaceans and fish, Mar.
- * Biol.,vol.27, pp. 75-88.

F008 IUC4

F009 13-11-2003

F020 4565

EOR

F002 37

F010 4.1

F004 7

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. Since
- * significant evaporative losses of lower hydrocarbons may have occurred,
- * the LC50 values are expected to be lower than those cited. These data are
- also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by API crude oil task group as '3', not reliable.

F020 4566

EOR

F002 37

```
F010 4.1
F0047
F005 RS
F006 The results were as follows:
    For Kuwait crude: 48-hour LC50 = 15 000 mg/l, 96-hour LC50 = 9400 mg/l
    For South Louisiana crude: 48-hour LC50 = 5000 mg/l
       96-hour LC50 = 3700 mg/l
F007 The results were as follows:
    For Kuwait crude: 48-hour LC50 = 15 000 mg/l, 96-hour LC50 = 9400 mg/l
    For South Louisiana crude: 48-hour LC50 = 5000 mg/l
       96-hour LC50 = 3700 mg/l
F020 4564
EOR
F002 37
F010 4.1
F0047
F005 SO
F006 CONCAWE Bruxelles
F007 CONCAWE Bruxelles
F008 IUC4
F009 09-10-2003
F020 4573
EOR
F002 37
F010 4.1
F004 8
F005 ME
F006 The test species was the Rainbow Trout. A water-soluble fraction (WSF) of
   the test substance was prepared by adding crude oil to water at a
   concentration of 12.5 ml/l and stirring for 2 hours. After settling for
   72 hours, groups of 5 fish
F007 The test species was the Rainbow Trout. A water-soluble fraction (WSF) of
   the test substance was prepared by adding crude oil to water at a
   concentration of 12.5 ml/l and stirring for 2 hours. After settling for
   72 hours, groups of 5 fish were exposed to solutions containing 20, 30,
   40 and 50% WSF. Three series of tests were run:
      (a) with closed vessels
      (b) with open vessels, and
      (c) with aerated vessels.
F020 4567
EOR
F002 37
F010 4.1
```

F006 Lockhart, W.L., Danell, R.W. and Murray, D.A. (1987)

- ** Acute toxicity bioassays with petroleum products: influence of exposure
- * conditions.

F004 8 F005 RE

- ** In: Oil in Freshwater: Chemistry, Biology, Counter-measure Technology,
- * Vandermeulen, J.H. and Hrud

F007 Lockhart, W.L., Danell, R.W. and Murray, D.A. (1987)

- ** Acute toxicity bioassays with petroleum products: influence of exposure
- * conditions.

```
** In: Oil in Freshwater: Chemistry, Biology, Counter-measure Technology,
```

* Vandermeulen, J.H. and Hrudey, S.E. (Eds), pp. 335-344, Pergamon Press.

F008 IUC4

F009 13-11-2003

F020 4569

EOR

F002 37

F010 4.1

F0048

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- These data are ranked by

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by API crude oil task group as '2', reliable with
- * restrictions.

F020 4570

EOR

F002 37

F010 4.1

F004 8

F005 RS

F006 The results were as follows:

```
* 48-hour LC50 (open vessel) = 10.4 mg/l
```

** 48-hour LC50 (closed vessel) = 11.6 mg/l

**

- ** based on measurements of dissolved hydrocarbons made at the
- ** beginning and end of the 48-hour period. No fish died

F007 The results were as follows:

** 48-hour LC50 (open vessel) = 10.4 mg/l ** 48-hour LC50 (closed vessel) = 11.6 mg/l

**

- ** based on measurements of dissolved hydrocarbons made at the
- ** beginning and end of the 48-hour period. No fish died in the aerated
- * vessels, and hydrocarbons were undetectable in solution at the 48-hour
- * time point in these studies.

F020 4568

EOR

F002 37

F010 4.1

F004 8

F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

F008 IUC4

F009 09-10-2003

F020 4574

EOR

F002 37

F010 4.2

```
F004 1
F005 ME
F006 LL 50 at 96 hr calculated maximum likelihood analysis based
** on D.J. Finney, 1971
F007 LL 50 at 96 hr calculated maximum likelihood analysis based
** on D.J. Finney, 1971
F020 4575
EOR
F002 37
F010 4.2
F004 1
F005 RE
F006 ExxonMobil Biomedical Sciences Inc. (2002)
** Mysid - acute toxicity test: Study No. 121260, test
** substance MRD-01-02. 2002.
** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.
F007 ExxonMobil Biomedical Sciences Inc. (2002)
** Mysid - acute toxicity test: Study No. 121260, test
** substance MRD-01-02. 2002.
** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.
F020 4576
EOR
F002 37
F010 4.2
F004 1
F005 RS
F006 Mortality (no. of deaths/treatment) at 96 hrs: 0, 2, 2, 1,
** 15 and 20, respectively for control, 0.14, 0.28, 1.4, 3.5,
** and 11 mg/l treatments.
** 96-hr LL50 = 2.7 mg/l, 95% C.I: 2.2-3.6 mg/l (as actual
** loading rate)
** Results are quoted in term
F007 Mortality (no. of deaths/treatment) at 96 hrs: 0, 2, 2, 1,
** 15 and 20, respectively for control, 0.14, 0.28, 1.4, 3.5,
** and 11 mg/l treatments.
** 96-hr LL50 = 2.7 mg/l, 95% C.I: 2.2-3.6 mg/l (as actual
** loading rate)
** Results are quoted in terms of 50% Lethal Loading (LL50),
** the loading rate of test substance resulting in 50%
   mortality of the test species exposed to the WAF.
        Measured BTEX (mg/l) in test treatment (mg/l load)
** Day
                                              0.28
                                                                             3.5
                                                                                             11
               Control
                              0.14
                                                              1.4
   0 (new) ND ND
                       ND
                             0.049 0.167 0.500
** 1 (old) ND
                ND
                       ND
                             0.0118 0.053 0.239
** 3 (new) 0.047 ND
                       0.003 0.0586 0.203 NA
** 4 (old) ND
                ND
                       ND
                             0.017 0.0939 NA
** ND=not detected, NA=not analyzed due to 100% mortality
** The practical quantitation limit was 0.003 mg/l. Measured
** soluble concentrations of BTEX may have been reduced by the
** presence of brine shrimp in the test chambers
F020 4577
```

EOR

```
F002 37
F010 4.2
```

F004 1

F005 TC

F006 Test species: Juvenile Mysids used in the test were 1day old and were

- * obtained from A.K. Siewers, Santa Cruz, CA, USA; the Mysids were
- * acclimated for 3 days under laboratory
- ** conditions in seawater supplied by the organism vendor and
- ** fed fro

F007 Test species: Juvenile Mysids used in the test were 1day old and were

- * obtained from A.K. Siewers, Santa Cruz, CA, USA; the Mysids were
- * acclimated for 3 days under laboratory
- ** conditions in seawater supplied by the organism vendor and
- ** fed from in-house cultures of Artemia salina nauplii that
- ** were less than 24 hours old. Test organisms were fed brine
- ** shrimp once per day during the study.

*

- ** Test System: Individual water accommodated fractions (WAFs)
- ** were prepared for each test level. New test solutions were
- ** prepared for each 24-hour interval throughout the duration
- ** of the test. The control and dilution water was natural
- ** seawater collected from Manasquan Inlet, NJ, (NJDEP
- ** designated collection site) with a salinity of 33% and
- ** passed through 0.45 um filters prior to use.

**

- ** Based on results of range-finding tests for a similar
- ** petroleum product, actual loading rates of 0, 0.14, 0.28,
- ** 1.4, 3.5 and 11 mg/l of crude oil to water were used to
- ** prepare test solutions for definitive toxicity tests. Test
- ** substance, added volumetrically, was mixed for each
- ** individual treatment in dilution water for 24 hours in
- ** appropriate-sized, stoppered containers with approximately
- ** 20% vortex and less than 10% headspace volume. The WAFs were allowed to
- * settle for 1-2 hours prior to drawing off the aqueous solutions for
- testing. A new WAF was prepared 24 hours prior to each renewal. Water
- * quality (temperature, pH, salinity, and dissolved oxygen) measurements
- * were recorded daily on the new solutions. Temperature, pH, and dissolved
- * oxygen measurements were recorded on the old solutions (composite of
- replicates).

**

- ** Test conditions: Two replicate chambers per treatment were
- ** tested. Each replicate contained ten mysids. Replicate
- ** chambers were 500 ml glass jars with Teflon screw lids,
- ** containing approximately 450 ml of test solution with no
- ** headspace. All test and control solutions were replenished
- ** daily. Renewals were accomplished by transferring the mysids to fresh
- * solution in a second set of test chambers on days 1, 2 and 3.

*

- ** Light: 16-hour light per day and a daylight intensity that
- ** ranged from 494 to 720 Lux.

**

** Test temperature: 11.9°C (S.D.=0.2).

**

```
** Water chemistry: Dissolved oxygen ranged from 6.4 - 7.0

** mg/l; pH ranged from 8.1 - 8.5; salinity ranged from 32 - 34 ppt.

**

** Freshly prepared and old WAF test solutions were analyzed by GC-FID for

* concentrations of BTEX.

F020 4578

EOR

F002 37

F010 4.2

F004 2

F005 ME
```

F006 LL 50 at 96 hr calculated maximum likelihood analysis based

** on D.J. Finney, 1971

F007 LL 50 at 96 hr calculated maximum likelihood analysis based

** on D.J. Finney, 1971

F020 4579

EOR

F002 37

F010 4.2

F004 2

F005 RE

F006 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Mysid acute toxicity test: Study No. 101160, test
- ** substance MRD-01-01.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F007 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Mysid acute toxicity test: Study No. 101160, test
- ** substance MRD-01-01.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F020 4580

EOR

F002 37

F010 4.2

F004 2

F005 RS

F006 Mortality (no. of deaths/treatment) at 96 hrs: 1, 2, 3, 6,

- ** 20 and 20, respectively for control, 0.6, 1.7, 3.6, 8.3 and
- ** 21 mg/l treatments.
- ** $96-hr\ LL50 = 4.1\ mg/I, 95\%\ C.I: 3.3-5.3\ mg/I\ (as\ actual$
- ** loading rate)
- ** Results are quoted in terms o

F007 Mortality (no. of deaths/treatment) at 96 hrs: 1, 2, 3, 6,

- ** 20 and 20, respectively for control, 0.6, 1.7, 3.6, 8.3 and
- ** 21 mg/l treatments.
- ** $96-hr\ LL50 = 4.1\ mg/I, 95\%\ C.I: 3.3-5.3\ mg/I\ (as\ actual$
- ** loading rate)
- ** Results are quoted in terms of 50% Lethal Loading (LL50),
- ** the loading rate of test substance resulting in 50%
- ** mortality of the test species exposed to the WAF.
- ** Measured BTEX (mg/l) in test treatment (mg/l load)
- ** Day Control 0.6 1.7 3.6 8.3 21
- ** 0 (new) ND ND ND ND 0.072 0.247
- ** 1 (old) ND ND ND ND ND 0.057

```
** 3 (new) ND ND ND 0.019 NA NA

** 4 (old) ND ND ND 0.023 NA NA
```

- ** ND=not detected, NA=not analyzed due to 100% mortality
- ** The practical quantitation limit was 0.003 mg/l. Measured
- ** soluble concentrations of BTEX may have been reduced by the
- ** presence of brine shrimp in the test chambers

F020 4581

EOR

F002 37

F010 4.2

F004 2

F005 TC

F006 Test species: Juvenile Mysids used in the test were 1day old

- ** and were obtained from A.K. Siewers, Santa Cruz, CA, USA;
- ** the Mysids were acclimated for 3 days under laboratory
- ** conditions in seawater supplied by the organism vendor and
- ** fed fro

F007 Test species: Juvenile Mysids used in the test were 1day old

- ** and were obtained from A.K. Siewers, Santa Cruz, CA, USA;
- ** the Mysids were acclimated for 3 days under laboratory
- ** conditions in seawater supplied by the organism vendor and
- ** fed from in-house cultures of Artemia salina nauplii that
- ** were less than 24 hours old. Test organisms were fed brine
- ** shrimp once per day during the study.

**

- ** Test System: Individual water accommodated fractions (WAFs)
- ** were prepared for each test level. New test solutions were
- ** prepared for each 24-hour interval throughout the duration
- ** of the test. The control and dilution water was natural
- ** seawater collected from Manasquan Inlet, NJ, (NJDEP
- ** designated collection site) with a salinity of 33% and
- ** passed through 0.45 um filters prior to use.

*

- ** Nominal loading rates of 0, 1.8, 19 and 105 mg/l were used
- ** to prepare test solutions for range-finding toxicity tests.
- ** Based on results of range-finding tests, actual loading
- ** rates of 0, 0.6, 1.7, 3.6, 8.3 and 21 mg/l of crude oil to
- ** water were used to prepare test solutions for definitive
- ** toxicity tests. Test substance, added volumetrically, was
- ** mixed for each individual treatment in dilution water for 24
- ** hours in appropriate-sized, stoppered containers with
- ** approximately 20% vortex and less than 10% headspace volume.
- ** The WAFs were allowed to settle for 1-2 hours prior to
- ** drawing off the aqueous solutions for testing. A new WAF was
- ** prepared 24 hours prior to each renewal. Water quality
- ** (temperature, pH, salinity, and dissolved oxygen)
- ** measurements were recorded daily on the new solutions.
- ** Temperature, pH, and dissolved oxygen measurements were
- ** recorded on the old solutions (composite of replicates).

*

- ** Test conditions: Two replicate chambers per treatment were
- ** tested. Each replicate contained ten mysids. Replicate

- ** chambers were 500 ml glass jars with Teflon® screw lids,
- ** containing approximately 450 ml of test solution with no
- ** headspace. All test and control solutions were replenished
- ** daily. Renewals were accomplished by transferring the mysids
- ** to fresh solution in a second set of test chambers on days
- ** 1, 2 and 3.
- ** Light: 16-hour light per day and a daylight intensity that
- ** ranged from 494 to 720 Lux.
- ** Test temperature: 12.3°C (S.D.=0.1).
- ** Water chemistry: Dissolved oxygen ranged from 6.4 7.0
- ** mg/l; pH ranged from 8.1 8.5; salinity ranged from 32 33
- ** ppt
- ** Freshly prepared and old WAF test solutions were analyzed by
- ** GC-FID for concentrations of BTEX.

F020 4582

EOR

F002 37

F010 4.2

F004 3

F005 ME

F006 The test species was the Brown Shrimp. Groups of 20 shrimps were exposed

- * to nominal concentrations in the range 17 to 3400 mg/l of each crude oil
- * in sea water. After addition of the crude oil, the solutions were
- * stirred at a constant rate

F007 The test species was the Brown Shrimp. Groups of 20 shrimps were exposed

- * to nominal concentrations in the range 17 to 3400 mg/l of each crude oil
- * in sea water. After addition of the crude oil, the solutions were
- * stirred at a constant rate using a shielded stirrer. Crude oil solutions
- * were renewed after 48 hours. Mortalities were recorded daily.

F020 4583

EOR

F002 37

F010 4.2

F004 3

F005 RE

F006 Franklin, F.L. and Lloyd, R.(1982)

- ** The toxicity of twenty-five oils in relation to the MAFF dispersant
- * tests,
- ** Fisheries Research Technical Report No. 70, 1982
- ** Ministry of Agriculture, Fisheries and Food, Lowestoft.

F007 Franklin, F.L. and Lloyd, R.(1982)

- ** The toxicity of twenty-five oils in relation to the MAFF dispersant
- * tests
- ** Fisheries Research Technical Report No. 70, 1982
- ** Ministry of Agriculture, Fisheries and Food, Lowestoft.

F008 IUC4

F009 13-11-2003

F020 4585

EOR

F002 37

F010 4.2

F004 3

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- Since no analytical dete

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * Since no analytical determinations were reported, these data are ranked
- * by API crude oil task group as '2', reliable with restrictions.

F020 4586

EOR

F002 37

F010 4.2

F0043

F005 RS

F006 The lowest LC50 value was obtained for Brent crude and the highest for

- * Thistle crude. Other North Sea crude oils tested were from the Argyll,
- * Auk, Beryl, Claymore, Ekofisk, Forties, Montrose, Murchison and Piper
- * fields.

F007 The lowest LC50 value was obtained for Brent crude and the highest for

- * Thistle crude. Other North Sea crude oils tested were from the Argyll,
- * Auk, Beryl, Claymore, Ekofisk, Forties, Montrose, Murchison and Piper
- * fields.

F020 4584

EOR

F002 37

F010 4.2

F004 3

F005 SO

F006 CONCAWE Bruxelles

**

**

F007 CONCAWE Bruxelles

**

**

F008 IUC4

F009 09-10-2003

F020 4606

EOR

F002 37

F010 4.2

F004 4

F005 ME

F006 The test species was the Brown Shrimp. Groups of 20 shrimps were exposed

- * $\,$ to nominal concentrations in the range 17 to 3400 mg/l of each crude oil
- * in sea water. After addition of the crude oil, the solutions were
- * stirred at a constant rate

F007 The test species was the Brown Shrimp. Groups of 20 shrimps were exposed

- * to nominal concentrations in the range 17 to 3400 mg/l of each crude oil
- * in sea water. After addition of the crude oil, the solutions were
- * stirred at a constant rate using a shielded stirrer. Crude oil solutions
- * were renewed after 48 hours. Mortalities were recorded daily.

F020 4587

EOR

F002 37

F010 4.2

F004 4

F005 RE

F006 Franklin, F.L. and Lloyd, R.(1982)

- ** The toxicity of twenty-five oils in relation to the MAFF dispersant
- * tests,
- ** Ministry of Agriculture, Fisheries and Food, Lowestoft. Fisheries
- * Research Technical Report No. 70, 1982.

F007 Franklin, F.L. and Lloyd, R.(1982)

- ** The toxicity of twenty-five oils in relation to the MAFF dispersant
- * tests,
- ** Ministry of Agriculture, Fisheries and Food, Lowestoft. Fisheries
- * Research Technical Report No. 70, 1982.

F020 4590

EOR

F002 37

F010 4.2

F004 4

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * Since no analytical dete

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * Since no analytical determinations were reported, these data are ranked
- * by API crude oil task group as '2', reliable with restrictions.

F020 4589

EOR

F002 37

F010 4.2

F004 4

F005 RS

F006 The lowest LC50 value was obtained for Abu Dhabi crude and the highest

- * for Iranian Light crude. Other crude oils tested were from Libya, Saudi
- * Arabia, Nigeria, Kuwait, Iraq and Iran (heavy crude).

F007 The lowest LC50 value was obtained for Abu Dhabi crude and the highest

- * for Iranian Light crude. Other crude oils tested were from Libya, Saudi
- * Arabia, Nigeria, Kuwait, Iraq and Iran (heavy crude).

F020 4588

EOR

F002 37

F010 4.2

F004 4

F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

F008 IUC4

F009 09-10-2003

F020 4607

FOR

F002 37

F010 4.2

F004 5

F005 ME

F006 Dispersions of oil in water at 5 concentrations in the range 10 to 100

- * mg/l were prepared by shaking the constituents together in closed jars
- * for 5 minutes using a
- ** reciprocating table. Groups of 10 daphnids were exposed to the resulting
- * 50

F007 Dispersions of oil in water at 5 concentrations in the range 10 to 100

- * mg/l were prepared by shaking the constituents together in closed jars
- * for 5 minutes using a
- ** reciprocating table. Groups of 10 daphnids were exposed to the resulting
- * solutions. Tests were run in open vessels.

F020 4591

EOR

F002 37

F010 4.2

F004 5

F005 RE

F006 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)
- * Conference on Environmental Sciences and Sustainable Development,
- * University of Sh

F007 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)
- * Conference on Environmental Sciences and Sustainable Development,
- * University of Sheffield, April 1991.

F020 4593

EOR

F002 37

F010 4.2

F004 5

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- Since significant evapor

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * Since significant evaporative losses of lower hydrocarbons may have
- * occurred, the LC50 values are expected to be lower than those cited.
- These data are ranked by API crude oil task group as '3', not reliable.

F020 4594

EOR

F002 37

F010 4.2

F004 5

F005 RS

F006 The results were as follows: 24-hour EC50 = 36 mg/l. As the tests were

- * run in open vessels, significant evaporative losses of lower hydrocarbons
- * will have occurred
- ** and hence the measured EC50 value underestimates the true ecotoxicity.

F007 The results were as follows: 24-hour EC50 = 36 mg/l. As the tests were

- * run in open vessels, significant evaporative losses of lower hydrocarbons
- * will have occurred
- ** and hence the measured EC50 value underestimates the true ecotoxicity.

F020 4592

FOR

F002 37

F010 4.2

F004 5

F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

F008 IUC4

F009 09-10-2003

F020 4608

EOR

F002 37

F010 4.2

F004 6

F005 ME

F006 Crude oil was added to water at 8 concentrations in the range 10 to 100

- * mg/l in open vessels fitted with shielded propeller stirrers. A constant
- * rate of stirring was
- ** maintained during the 24-hour exposure period to groups of 10 daphnids.

*

F007 Crude oil was added to water at 8 concentrations in the range 10 to 100

- * mg/l in open vessels fitted with shielded propeller stirrers. A constant
- rate of stirring was
- ** maintained during the 24-hour exposure period to groups of 10 daphnids.

**

F020 4597

EOR

F002 37

F010 4.2

F004 6

F005 RE

F006 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)
- * Conference on Environmental Sciences and Sustainable Development,
- * University of Sh

F007 Westlake, G.E.(1991

- ** Aquatic test media preparation for low solubility materials, Proceedings
- * of the Society of Environmental Toxicology and Chemistry (SETAC)
- * Conference on Environmental Sciences and Sustainable Development,
- University of Sheffield, April 1991.

F020 4596

EOR

F002 37

F010 4.2

F004 6

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- Since significant evapor

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * Since significant evaporative losses of lower hydrocarbons may have
- * occurred, the LC50 values are expected to be lower than those cited.
- * These data are ranked by API crude oil task group as '3', not reliable.

F020 4595

FOR

F002 37

F010 4.2

F004 6

F005 RS

F006 The results were as follows: 24-hour EC50 = 42 mg/l. As the tests were

- * run in open vessels, significant evaporative losses of lower hydrocarbons
- will have occurred
- ** and hence the measured EC50 value underestimates the true ecotoxicity.

F007 The results were as follows: 24-hour EC50 = 42 mg/l. As the tests were

- * run in open vessels, significant evaporative losses of lower hydrocarbons
- * will have occurred
- ** and hence the measured EC50 value underestimates the true ecotoxicity.

F020 4598

EOR

F002 37

F010 4.2

F004 6

F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

F008 IUC4

F009 09-10-2003

F020 4609

EOR

F002 37

F010 4.2

F004 7

F005 ME

F006 Tests were carried out in polypropylene containers using water

- * accommodated fractions prepared by physical dispersion. These were
- renewed daily, and kept tightly covered with teflon sheets. Loading rates
- of 11.6, 34.3, 99.1, 284 and 862 mg/

F007 Tests were carried out in polypropylene containers using water

- * accommodated fractions prepared by physical dispersion. These were
- * renewed daily, and kept tightly covered with teflon sheets. Loading rates
- * of 11.6, 34.3, 99.1, 284 and 862 mg/l were used in this study. The test
- * species was the kelp forest mysid, Holmesimysis costata.

**

F020 4599 **EOR** F002 37 F010 4.2 F0047 F005 RE F006 Bragin, G.E., Clark, J.R. and Pace, C.B.(1994) ** Comparison of physically and chemically dispersed crude oil toxicity under continuous and spiked exposure scenarios, Marine Spill Response Corporation, Washington D.C., MSRC Technical Report Se F007 Bragin, G.E., Clark, J.R. and Pace, C.B.(1994) ** Comparison of physically and chemically dispersed crude oil toxicity under continuous and spiked exposure scenarios, Marine Spill Response Corporation, Washington D.C., MSRC Technical Report Series 94-015, 45p. F008 IUC4 F009 13-11-2003 F020 4601 **EOR** F002 37 F010 4.2 F004 7 F005 RM F006 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by F007 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without restrictions. F020 4602 **EOR** F002 37 F010 4.2 F0047 F005 RS F006 A lethal loading rate concentration, LL50 (96h) of 39.5 mg/l was calculated from data presented in the original publication after consultation with the main author. Weight percent distribution for the crude oil components with BP up to 425 F007 A lethal loading rate concentration, LL50 (96h) of 39.5 mg/l was calculated from data presented in the original publication after consultation with the main author. Weight percent distribution for the crude oil components with BP up to 425 °F are: paraffins: 68.56%; aromatics: 15.69 and naphthenes: 11.86%. F020 4600 **EOR** F002 37 F010 4.2

F004 7 F005 SO

F006 CONCAWE Bruxelles

F007 CONCAWE Bruxelles

**

F008 IUC4

F009 09-10-2003

F020 4610

EOR

F002 37

F010 4.2

F004 8

F005 ME

F006 Tests were carried out in polypropylene containers using water

- * accommodated fractions prepared by physical dispersion. These were
- * renewed daily and kept tightly covered with teflon sheets. Loading rates
- * of 340, 695, 1550, 3470 and 7740 mg/

F007 Tests were carried out in polypropylene containers using water

- * accommodated fractions prepared by physical dispersion. These were
- * renewed daily and kept tightly covered with teflon sheets. Loading rates
- * of 340, 695, 1550, 3470 and 7740 mg/l were used in this study. The test
- * species was the estuarine mysid, Mysodopsis bahia.

**

F020 4603

EOR

F002 37

F010 4.2

F004 8

F005 RM

F006 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by

F007 These data are included to provide supporting evidence of the expected

- * cumulative toxicity of the soluble components in crude oil. These data
- * are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9.
- * These data are ranked by API crude oil task group as '1', reliable
- * without restrictions.

F020 4605

EOR

F002 37

F010 4.2

F004 8

F005 RS

F006 A lethal loading rate concentration LL50 (96h) of 618 mg/l was calculated

- * from data presented in the original publication after consultation with
- * the main author. . Weight percent distribution for the crude oil
- * components with BP up to 425

F007 A lethal loading rate concentration LL50 (96h) of 618 mg/l was calculated

- * from data presented in the original publication after consultation with
- * the main author. . Weight percent distribution for the crude oil
- * components with BP up to 425 °F are: paraffins: 68.56%; aromatics: 15.69
- and naphthenes: 11.86%.

F020 4604

EOR

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F002 37
F010 4.2
F0048
F005 SO
F006 CONCAWE Bruxelles
F007 CONCAWE Bruxelles
F008 IUC4
F009 09-10-2003
F020 4611
EOR
F002 37
F010 4.3
F004 1
F005 ME
F006 The method used was described by Chapman, G.A., D.L. Denton
   and J.M. Lazorchak. (1995).
** Statistical method
** Lethal Loading and Effect Loading: Norberg-King, T.J., A
** Linear Interpolation Method for Sublethal Toxicity: The
** Inhibition Concent
F007 The method used was described by Chapman, G.A., D.L. Denton
   and J.M. Lazorchak. (1995).
** Statistical method
** Lethal Loading and Effect Loading: Norberg-King, T.J., A
** Linear Interpolation Method for Sublethal Toxicity: The
** Inhibition Concentration (ICp) Approach (Version 2.0). July
** 1993. U.S. Environmental Protection Agency, Environmental
** Research Laboratory, Duluth MN.
F020 4612
EOR
F002 37
F010 4.3
F004 1
F005 RE
F006 Chapman, G.A., Denton, D.L. and Lazorchak, J.M. (1995)
** Short-Term Methods for Estimating the Chronic Toxicity of
** Effluents and Receiving Waters to West Coast Marine and
** Estuarine Organisms. EPA-600/R-95-136.
** US Environmental Protection Age
F007 Chapman, G.A., Denton, D.L. and Lazorchak, J.M. (1995)
** Short-Term Methods for Estimating the Chronic Toxicity of
** Effluents and Receiving Waters to West Coast Marine and
** Estuarine Organisms. EPA-600/R-95-136.
** US Environmental Protection Agency, Cincinnati, OH.
F008 IUC4
F009 28-08-2003
F020 4613
EOR
F002 37
F010 4.3
```

F004 1

F005 RE

F006 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Giant Kelp, Germination and Growth Test: Study No. 121274,
- ** test substance MRD-01-02. 2002.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F007 ExxonMobil Biomedical Sciences Inc. (2002)

- ** Giant Kelp, Germination and Growth Test: Study No. 121274,
- ** test substance MRD-01-02. 2002.
- ** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.

F020 4614

EOR

F002 37

F010 4.3

F004 1

F005 RS

F006 48 hour results, based on mg of test substance per liter of

** natural seawater

**

**	Germination	Tube Length	Germination	Tube Length			
**	LL50	EL50		NOEL	LOEL	NOEL	LOEL
**	(mg/l)	(mg/l)		(mg/l)	(mg/l)	(mg/l) (mg/l)	
**	122	122	52	146	18	52	

F007 48 hour results, based on mg of test substance per liter of

** natural seawater

**

**	Germination	Tube Length	Germination	Tube Length			
**	LL50	EL50		NOEL	LOEL	NOEL	LOEL
**	(mg/l)	(mg/l)		(mg/l)	(mg/l)	(mg/l) (mg/l)	
**	122	122	52	146	18	52	

** Analytical results

*

** Nominal (mg/l)

Measured Concentration (mg/l as BTEX)

*

**		Day 0	Day 2	
**	Control	none d	etected	none detected
**	18	0.542	0.4	78
**	52	1.82	1.23	3
**	146	5.91	3.2	7
**	415	15.2	5.8	8
**	1185	20.0	11.	3

** Practical Quantitation Limit (PQL) was approximately 0.0035

** mg/l (3.5ng/ml)

F020 4615

EOR

F002 37

F010 4.3

F004 1

F005 TC

F006 Individual test treatment solutions were prepared as Water

** Accommodated Fractions (WAFs). Actual loading levels of test substance in

- * natural seawater (dilution water) were 18 mg/l, 52 mg/l, 146 mg/l, 415
- * mg/l and 1185 mg/l. A control
- ** treat

F007 Individual test treatment solutions were prepared as Water

- ** Accommodated Fractions (WAFs). Actual loading levels of test substance in
- * natural seawater (dilution water) were 18 mg/l, 52 mg/l, 146 mg/l, 415
- * mg/l and 1185 mg/l. A control
- ** treatment consisting of natural seawater with no test
- ** substance was also prepared. The WAFs were prepared by
- ** adding the appropriate amount of test substance to natural
- ** seawater in glass aspirator bottles and stirring on magnetic stirplates
- * $\,$ for 24 hours. Natural seawater was filtered through a 0.45 μm filter,
- * then a 0.2 μm filter (collected from Manasquan Inlet, NJ, NJDEP
- * designated collection site).
- ** The salinity of the seawater was 34 ± 2 ppt.
- ** Ammonia (NH3 + NH4+) = 0 mg/l
- ** Nitrite (NO2-) = <0.3 mg/l
- ** Nitrate (N) = 1 mg/l.
- ** The mixtures were allowed to settle and cool to test
- ** temperature for 1.25 hour before removing the aqueous
- ** portions (WAFs) for testing. Samples of the WAFs prepared at each loading
- * level were taken on Day 0 and at termination from each treatment
- * (composite of replicates). The samples were analyzed for benzene,
- * toluene, ethylbenzene and o-xylene (BTEX), all hydrocarbons detected in
- * the entire chromatographic run were quantified versus the BTEX standards
- * and the reported results reflect the sum of all quantified compounds.
- * Samples were taken with no headspace in volatile organic analysis (VOA)
- * vials
- ** Test temperature was 14.0 (sd=0.3) °C.
- *
- ** The kelp sporophylls (reproductive blades) were received
- ** from A. K. Siewers, Santa Cruz, CA 95060, packed on ice at
- ** 7.5°C, the day after they were harvested. The sporophylls
- ** $\,$ were rinsed with 0.2 μm filtered seawater at 14°C and
- ** blotted dry with paper towels. They were then desiccated at 14°C for one
- * hour. The sporophylls were rinsed again with 0.2µm filtered seawater at
- * 14°C. Approximately 20 of the sporophylls were placed into a one liter
- glass beaker
- ** containing 800 ml of 0.2 µm filtered seawater at 14°C. The
- ** blades were left in the beaker for 30 minutes while the
- ** zoospores released. The blades were removed and the spore
- ** solution was allowed to settle for 20 minutes. 250 ml was
- ** poured from the top of the beaker, this was the spore stock.
- ** The zoospores were released from the sporophylls and
- ** $\,$ inoculated into test chambers within a two hour period. The zoospore
- * release was initiated within 24 hours of sporophyll harvest.
- ** Five replicate chambers for each dose treatment consisted of 500 ml size
- * glass jars sealed with Teflon® lined screw type lids, containing
- * approximately 450 ml of test solution to minimize headspace and prevent
- loss of volatile components.
- ** A pre-cleaned 2.5 cm x 7.6 cm glass slide was placed in the
- ** bottom of each jar prior to adding test solution. The

- ** initial concentration was approximately 7.5 x 103
- ** zoospores/ml in each replicate chamber. The spore stock
- ** density was determined as follows: 1 ml of the stock and 1
- ** ml of glacial acetic acid were added to 8 ml of 0.2 µm
- ** filtered seawater in a graduated cylinder. The solution
- ** was mixed and counts were performed using a hemacytometer at 100x. Each
- * test chamber, containing approximately 7500
- ** spores, was labeled to show the study number, loading level, replicate
- * and randomization number. Each test and control chamber were observed for
- * germination of the gametophyte spores and length of the embryonic
- * gametophyte germination tube after approximately 48 hrs. The following
- * procedure was used. The glass slide was removed from the jar and the
- * bottom of the slide was dried with a paper towel. A 24 x 50 mm cover
- * slideslip was placed onto the slide and the edges were dabbed.
- * Germination was considered successful if a germ tube was present on the
- * settled zoospore. Germination was considered unsuccessful if no germ
- tube was visible.
- ** 400x magnification was used to differentiate germination and measure the
- * tubes. A spore had to have a protuberance that extended at least one
- * spore diameter from the edge of the spore to be considered germinated.
- * The first 100 spores, which were easily identifiable, were counted while
- * moving the slide across the field of view of the microscope.
- ** Measurements were made by moving the slide and choosing the
- ** first 10 spores with straight germ tubes within the same
- ** focal plane of the microscope that were closest to the scale in the
- * ocular micrometer. The ocular micrometer was
- ** calibrated with a stage micrometer at 400x to assure
- ** accurate measurements. The micrometer was calibrated from 3 to 25 µm.
- * $\,$ The total length of the tube from the edge of the original spore membrane
- was measured.
- **
- ** Water quality (temperature, pH, dissolved oxygen, and
- ** salinity) measurements were made on each treatment on Day 0
- ** and at termination (composite of replicates). BTEX total
- ** concentration at termination was at least 80% of the initial
- concentration.

- ** The study was deemed acceptable. Mean control germination
- ** was > 70% in the controls. Mean germination tube length was > 10 μm in
- the controls.

F020 4616

EOR

F002 37

F010 4.3

F004 2

F005 ME

F006 Lethal Loading and Effect Loading: Norberg-King, T.J., A

- ** Linear Interpolation Method for Sublethal Toxicity: The
- ** Inhibition Concentration (ICp) Approach (Version 2.0). July
- ** 1993. U.S. Environmental Protection Agency, Environmental
- ** Research

F007 Lethal Loading and Effect Loading: Norberg-King, T.J., A

** Linear Interpolation Method for Sublethal Toxicity: The

```
** Inhibition Concentration (ICp) Approach (Version 2.0). July
** 1993. U.S. Environmental Protection Agency, Environmental
** Research Laboratory, Duluth MN.
F020 4617
EOR
F002 37
F010 4.3
F004 2
F005 RE
F006 Chapman, G.A., Denton, D.L. and Lazorchak, J.M. (1995)
** Short-Term Methods for Estimating the Chronic Toxicity of
** Effluents and Receiving Waters to West Coast Marine and
** Estuarine Organisms. EPA-600/R-95-136.
** US Environmental Protection Age
F007 Chapman, G.A., Denton, D.L. and Lazorchak, J.M. (1995)
** Short-Term Methods for Estimating the Chronic Toxicity of
** Effluents and Receiving Waters to West Coast Marine and
** Estuarine Organisms. EPA-600/R-95-136.
** US Environmental Protection Agency, Cincinnati, OH.
F008 IUC4
F009 28-08-2003
F020 4618
EOR
F002 37
F010 4.3
F004 2
F005 RE
F006 ExxonMobil Biomedical Sciences Inc. (2002)
** Giant Kelp, Germination and Growth Test: Study No. 101174,
** test substance MRD-01-01.
** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.
F007 ExxonMobil Biomedical Sciences Inc. (2002)
** Giant Kelp, Germination and Growth Test: Study No. 101174,
** test substance MRD-01-01.
** ExxonMobil Biomedical Sciences Inc. Annandale, NJ.
F020 4619
EOR
F002 37
F010 4.3
F004 2
F005 RS
F006 48 hour results, based on mg of test substance per liter of
   natural seawater (95%CI)
                                                            Tube Length LL50
                                                                                                         EL50
  Germination
                              Tube Length
                                           Germination
   NOEL
               LOEL
                              NOELLOEL
              311
                              130 23
                         53
                                           53 (497
F007 48 hour results, based on mg of test substance per liter of
   natural seawater (95%CI)
** Germination
                                                            Tube Length LL50
                                                                                                         EL50
                              Tube Length
                                             Germination
   NOEL
               LOEL
                              NOELLOEL
** 528
              311
                         53
                              130 23
                                         53 (497-558)
```

```
* (257-403)
```

** Analytical results

* Nominal (mg/l) Measured Concentration (mg/l as BTEX)

**

**		Day 0	Day 2
**	Control	none detecte	ed none detected
**	23	0.047	none detected
**	53	0.586	0.039
**	130	1.09	0.428
**	323	3.35	1.92
**	802	5.03	4.43

**

- ** Practical Quantitation Limit (PQL) was approximately 0.0035
- ** mg/l (3.5ng/ml)

*

**

- ** Spore concentration was 1000 spore/ml, instead of 7500
- ** spore/ml, as required. However total spore number per
- ** replicate was adequate sample size for determination of end
- ** points.

F020 4620

EOR

F002 37

F010 4.3

F004 2

F005 TC

F006 Individual test treatment solutions were prepared as Water

- ** Accommodated Fractions (WAFs). Actual loading levels of test
- ** substance in natural seawater (dilution water) were 23 mg/l,
- ** 53 mg/l, 130 mg/l, 323 mg/l and 802 mg/l. A control
- ** treatme

F007 Individual test treatment solutions were prepared as Water

- ** Accommodated Fractions (WAFs). Actual loading levels of test
- ** substance in natural seawater (dilution water) were 23 mg/l,
- ** $\,$ 53 mg/l, 130 mg/l, 323 mg/l and 802 mg/l. A control
- ** treatment consisting of natural seawater with no test
- ** substance was also prepared. The WAFs were prepared by
- ** adding the appropriate amount of test substance to natural
- ** seawater in glass aspirator bottles and stirring on
- ** magnetic stirplates for 24 hours. Natural seawater was
- ** filtered through a 0.45 μm filter, then a 0.2 μm filter
- ** (collected from Manasquan Inlet, NJ, NJDEP designated
- ** collection site). The salinity of the seawater was 34 ± 2
- ** ppt. The mixtures were allowed to settle and cool to test
- ** temperature for 1 hour before removing the aqueous portions
- ** (WAFs) for testing. Samples of the WAFs prepared at each
- ** loading level were taken on Day 0 and at termination from
- ** each treatment (composite of replicates). The samples were
- ** analyzed for benzene, toluene, ethylbenzene and o-xylene
- ** (BTEX), all hydrocarbons detected in the entire
- ** chromatographic run were quantified versus the BTEX

- ** standards and the reported results reflect the sum of all
- ** quantified compounds. Samples were taken with no headspace
- ** in volatile organic analysis (VOA) vials.
- ** Mean test temperature was 14.1 (sd=0.3) °C.

- ** The kelp sporophylls (reproductive blades) were received
- ** from A. K. Siewers, Santa Cruz, CA 95060, packed on ice at
- * 12.2°C, the day after they were harvested. The sporophylls
- ** were rinsed with 0.2 µm filtered seawater at 14°C and
- ** blotted dry with paper towels. They were then desiccated at
- ** 14°C for one hour. The sporophylls were rinsed again with
- ** 0.2µm filtered seawater at 14°C. Approximately 20 of the
- ** sporophylls were placed into a one liter glass beaker
- ** containing 800 ml of 0.2 µm filtered seawater at 14°C. The
- * blades were left in the beaker for 30 minutes while the
- ** zoospores released. The blades were removed and the spore
- ** solution was allowed to settle for 25 minutes. 250 ml was
- ** poured from the top of the beaker, this was the spore stock.
- ** The zoospores were released from the sporophylls and
- ** inoculated into test chambers within a two hour period. The
- ** zoospore release was initiated within 24 hours of sporophyll
- ** harvest.
- ** Five replicate chambers for each dose treatment consisted of
- ** 500 ml size glass jars sealed with Teflon® lined screw type
- ** lids, containing approximately 450 ml of test solution. A
- ** pre-cleaned 2.5 cm x 7.6 cm glass slide was placed in the
- ** bottom of each jar prior to adding test solution. The
- ** initial concentration was approximately 1.0 x 103
- ** zoospores/ml in each replicate chamber. The spore stock
- ** density was determined as follows: 1 ml of the stock and 1
- ** ml of glacial acetic acid were added to 8 ml of 0.2 μm
- ** filtered seawater in a graduated cylinder. The solution
- ** was mixed and counts were performed using a hemacytometer at
- * 100x. Each test chamber, containing approximately 1000
- ** spores, was labeled to show the study number, loading level,
- ** replicate and randomization number. Each test and control
- ** chamber was observed for germination of the gametophyte
- ** spores and length of the embryonic gametophyte germination
- * tube. The following procedure was used. The glass slide
- ** was removed from the jar, and the bottom of the slide was
- * dried with a paper towel. A 24 x 50 mm cover slideslip was
- ** placed onto the slide and the edges were dabbed.
- ** Germination was considered successful if a germ tube was
- ** present on the settled zoospore. Germination was considered
- ** unsuccessful if no germ tube was visible. 400x
- ** magnification was used to differentiate germination and
- ** measure the tubes. A spore had to have a protuberance that
- ** extended at least one spore diameter from the edge of the
- ** spore to be considered germinated. The first 100 spores,
- ** which were easily identifiable, were counted while moving
- ** the slide across the field of view of the microscope.
- ** Measurements were made by moving the slide and choosing the
- ** first 10 spores with straight germ tubes within the same

- ** focal plane of the microscope that were closest to the scale
- ** in the ocular micrometer. The ocular micrometer was
- ** calibrated with a stage micrometer at 400x to assure
- ** accurate measurements. The micrometer was calibrated from 3
- ** $\,$ to 25 $\mu m. \,$ The total length of the tube from the edge of the
- ** original spore membrane was measured.
- ** Water quality (temperature, pH, dissolved oxygen, and
- ** salinity) measurements were made on each treatment on Day 0
- ** and at termination (composite of replicates). BTEX total
- ** concentration at termination was at least 80% of the initial
- ** concentration.
- ** The study was deemed acceptable. Mean control germination
- ** was > 70% in the controls. Mean germination tube length was
- ** > 10 um in the controls.

F020 4621

EOR

F002 37

F010 4.3

F004 3

F005 ME

F006 Tests were run using:

- ** (a) a water-soluble fraction (WSF) of the test
- ** substance, and
- ** (b) the test substance in equilibrium with the
- ** algal suspension medium.
- ** To prepare the WSF, crude oil was added to the sterilized medium in

F007 Tests were run using:

- ** (a) a water-soluble fraction (WSF) of the test
- ** substance, and
- ** (b) the test substance in equilibrium with the
- ** algal suspension medium.
- ** To prepare the WSF, crude oil was added to the sterilized medium in the
- ratio 1:20, and was stirred in closed bottles for 12 hours. After a
- * 4-hour separation period the aqueous
- ** phase was separated and diluted for the tests. In the direct loading
- * tests, crude oil was applied to absorbent pads and these were held in the
- culture suspension. Analysis was done by gas chromatography, following
- * solvent extraction of the aqueous phase using n-pentane.

F020 4622

EOR

F002 37

F010 4.3

F004 3

F005 RE

F006 Gaur, J.P. and Singh, A.K. (1989)

- ** Comparative studies on the toxicity of petroleum oils and their aqueous
- * extracts towards Anabaena doliolum,
- ** Proc. Indian Acad. Sci. (Plant Sci.), vol. 99, pp. 459-466,

F007 Gaur, J.P. and Singh, A.K. (1989)

- ** Comparative studies on the toxicity of petroleum oils and their aqueous
- * extracts towards Anabaena doliolum,
- ** Proc. Indian Acad. Sci. (Plant Sci.), vol. 99, pp. 459-466,

F020 4624

EOR F002 37 F010 4.3 F004 3 F005 RM F006 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by F007 These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without restrictions. F020 4625 **EOR** F002 37 F010 4.3 F0043 F005 RS F006 The WSF method gave a mean 15-day EC50 of 9.06 mg/l, and ** the direct loading (or whole oil) method gave a 15-day EC50 ** of 5.73 mg/l, both based on measured dissolved hydrocarbon concentrations in the aqueous phase. F007 The WSF method gave a mean 15-day EC50 of 9.06 mg/l, and ** the direct loading (or whole oil) method gave a 15-day EC50 ** of 5.73 mg/l, both based on measured dissolved hydrocarbon concentrations in the aqueous phase. F020 4623 **EOR** F002 37 F010 4.3 F0043 F005 SO F006 CONCAWE Bruxelles F007 CONCAWE Bruxelles F008 IUC4 F009 09-10-2003 F020 4626 **EOR** F002 37 F010 4.9 F004 1

F006 CONCAWE (1992)

** Fcotoxicological test

- ** Ecotoxicological testing of petroleum products: test methodology
- ** Report No. 92/56

F005 RE

** CONCAWE, Brussels.

F007 CONCAWE (1992)

- ** Ecotoxicological testing of petroleum products: test methodology
- ** Report No. 92/56
- ** CONCAWE, Brussels.

F008 IUC4

F009 13-11-2003

F020 4628

EOR

F002 37

F010 4.9

F004 1

F005 RE

F006 GESAMP (1989)

- ** Reports and Studies No. 35, Annex 9, Advice for aquatic toxicity testing
- * of substances or of mixtures containing compounds of low solubility
- ** International Maritime Organization (IMO), London

F007 GESAMP (1989)

- ** Reports and Studies No. 35, Annex 9, Advice for aquatic toxicity testing
- * of substances or of mixtures containing compounds of low solubility
- ** International Maritime Organization (IMO), London

F008 IUC4

F009 13-11-2003

F020 4629

EOR

F002 37

F010 4.9

F004 1

F005 RE

F006 Whitehouse, P. and Mallet, M. (1983)

- ** Aquatic toxicity testing for notification of new substances. An advisory
- * document for dealing with "difficult" substances
- ** Water Research Centre (WRC), Medmenham UK.

F007 Whitehouse, P. and Mallet, M. (1983)

- ** Aquatic toxicity testing for notification of new substances. An advisory
- * document for dealing with "difficult" substances
- ** Water Research Centre (WRC), Medmenham UK.

F008 IUC4

F009 13-11-2003

F020 4630

EOR

F002 37

F010 4.9

F004 1

F005 RM

F006 For the assessment of the ecotoxicity of poorly water soluble mixtures of

- * hydrocarbons as found in petroleum products, it is now generally accepted
- * that results should be expressed in terms of the "loading rate". The
- * "loading rate" may be

F007 For the assessment of the ecotoxicity of poorly water soluble mixtures of

- * hydrocarbons as found in petroleum products, it is now generally accepted
- * that results should be expressed in terms of the "loading rate". The
- * "loading rate" may be defined as the amount of the product which must be
- equilibrated with the aqueous test medium in order to produce a specified
- level of effect. Studies in which the results are expressed in terms of

- * the measured concentrations of hydrocarbons in dilutions of "water
- * soluble fractions (WSF)" do not allow the ecotoxicity of a product to be
- * expressed in terms of the amount of that product required to produce a
- * particular effect and, therefore, such results are not comparable to
- * results
- ** obtained with other substances.

F020 4627

EOR

F002 37

F010 4.9

F004 1

F005 RM

F006 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '1',
- reliable without restrictions.

F007 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '1',
- reliable without restrictions.

F020 4631

EOR

F002 37

F010 4.9

F004 1

F005 SO

F006 CONCAWE Bruxelles

**

F007 CONCAWE Bruxelles

**

F008 IUC4

F009 09-10-2003

F020 4644

EOR

F002 37

F010 4.9

F004 2

F005 RE

F006 Hoffman, D.J. and Albers, P.H. (1984)

- ** Evaluation of potential embryotoxicity and teratogenicity of 42
- * herbicides, insecticides and petroleum contaminants to mallard eggs
- ** Arch. Environ. Contam. Toxicol., vol. 13, pp. 15-27.

F007 Hoffman, D.J. and Albers, P.H. (1984)

- ** Evaluation of potential embryotoxicity and teratogenicity of 42
- * herbicides, insecticides and petroleum contaminants to mallard eggs
- ** Arch. Environ. Contam. Toxicol., vol. 13, pp. 15-27.

F008 IUC4

F009 13-11-2003

F020 4633

EOR

F002 37

F010 4.9

F004 2

F005 RM

F006 Crude oil from 4 locations was applied externally at different loadings

- * to batches of 30 fertilized eggs of mallard ducks on day 3 of incubation
- at 37.5 °C. Eggs were candled each day to determine mortality, and dead
- * embryos examined for ab

F007 Crude oil from 4 locations was applied externally at different loadings

- * to batches of 30 fertilized eggs of mallard ducks on day 3 of incubation
- at 37.5 °C. Eggs were candled each day to determine mortality, and dead
- * embryos examined for abnormalities. On day 18 of incubation, surviving
- * embryos were examined for external malformations. The observed LD50
- * values for crude oils from Kuwait, Prudhoe Bay, South Louisiana and Texas
- * $\,$ were 2.2, 8.3, 1.3 and 5.5 $\mu\text{l/egg},$ respectively. The South Louisiana and
- * Texas crudes both produced significantly
- ** reduced growth, both above and below the LD50 value; preformed embryos
- * were also found with both these crudes.

F020 4632

FOR

F002 37

F010 4.9

F004 2

F005 RM

F006 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- reliability not assigned

F007 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- * reliability not assigned

F020 4634

EOR

F002 37

F010 4.9

F004 3

F005 RE

F006 Forns, J.M. (1977)

- ** The effects of crude oil on larvae of lobster, Homarus americanus.
- ** In: Proceedings of the API-EPA-US Coast Guard 1977 Oil Spill Conference
- * Prevention Behaviour, Control, Clean-up.
- ** American Petroleum Institute, Publ

F007 Forns, J.M. (1977)

- ** The effects of crude oil on larvae of lobster, Homarus americanus.
- ** In: Proceedings of the API-EPA-US Coast Guard 1977 Oil Spill Conference
- * Prevention Behaviour, Control, Clean-up.
- ** American Petroleum Institute, Publication No. 4284, pp. 569-573.

F008 IUC4

F009 13-11-2003

F020 4636

EOR

F002 37

F010 4.9

F004 3

F005 RM

F006 The effects of emulsified South Louisiana crude oil on the development of

- * American Lobster, Homarus americanus, was investigated during the
- * development of the first four larval stages over a 15-day period.
- * Hatched larvae were exposed to co

F007 The effects of emulsified South Louisiana crude oil on the development of

- * American Lobster, Homarus americanus, was investigated during the
- * development of the first four larval stages over a 15-day period.
- Hatched larvae were exposed to concentrations of 0, 0.1 and 1.0 ppm crude
- * oil, six times per day for periods ranging from 0.8 to 5.6 minutes, using
- * a flow-through system. The tests showed that 1.0 ppm crude oil was a
- * sub-lethal concentration for the lobster larvae and that no significant
- * effects were found at 0.1 ppm. At 0.1 ppm crude oil, the survival rate
- * of the larvae was comparable with that of the controls, but at 1.0 ppm
- * the survival value was about 50% that of the control larvae. Other
- * effects observed at 1.0 crude oil included: (a) lethargy, reduced
- * feeding and lack of the characteristic aggression of the control
- * animals, (b) an increase in development time from 12 to 15 days, and (c)
- * a change in the pigmentation of the larvae from the normal pale blue,
- * almost transparent, color to a sharp red appearance.

F020 4635

FOR

F002 37

F010 4.9

F004 3

F005 RM

F006 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- reliability not assigned

F007 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- * reliability not assigned

F020 4637

EOR

F002 37

F010 4.9

F004 3

F005 SO

F006 CONCAWE Bruxelles

**

F007 CONCAWE Bruxelles

**

F008 IUC4

F009 09-10-2003

F020 4645

EOR

F002 37

F010 4.9

F004 4

F005 RE

F006 Burks, S.L. (1980)

- ** Effects of oil on aquatic organisms. A review of selected literature
- ** American Petroleum Institute, Publication No. 29-30299, API, Washington DC F007 Burks, S.L. (1980)
- ** Effects of oil on aquatic organisms. A review of selected literature
- ** American Petroleum Institute, Publication No. 29-30299, API, Washington DC

F008 IUC4

F009 13-11-2003

F020 4639

EOR

F002 37

F010 4.9

F004 4

F005 RM

F006 Burks has extensively reviewed the effects of crude and refined oils on

- * organisms found in fresh and sea water. He noted that where spillages
- * occur the non-mobile species suffer the greatest mortality, whereas fish
- * species can often escap

F007 Burks has extensively reviewed the effects of crude and refined oils on

- * organisms found in fresh and sea water. He noted that where spillages
- * occur the non-mobile species suffer the greatest mortality, whereas fish
- * species can often escape from the affected region. The extent of the
- initial mortality depends on the chemical nature of the oil, the
- * location, and the physical conditions, particularly the temperature and
- * wind velocity. Most affected freshwater and marine communities recover
- * from the effects of an oil spill within a year. The occurrence of
- * biogenic hydrocarbons in the world's oceans is well recorded. They have
- * the characteristic isoprenoid structure, and measurements made in water
- * columns indicate a background concentration of 1.0 to 10 µl/l. The
- * higher molecular weight materials are dispersed as particles, with the
- * highest concentrations of about 20 μl/l occurring in the top 3 mm layer
- * of water.
- ** A wide variation in the response of organisms to oil exposures has been
- * noted. The larvae of fish and crustaceans appear to be most susceptible
- to the water-soluble fraction of crude oil. Exposures of plankton and
- algae have indicated that certain species of diatoms and green algae are
- inhibited, whereas microflagellates are not.
- ** For the most part, molluscs and most intertidal worm species appear to be
- * tolerant of oil contamination.

F020 4638

EOR

F002 37

F010 4.9

F004 4

F005 RM

F006 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- reliability not assigned

F007 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- reliability not assigned

F020 4640

EOR

F002 37

F010 4.9

F004 4

F005 SO

F006 CONCAWE Bruxelles

**

F007 CONCAWE Bruxelles

**

F008 IUC4

F009 09-10-2003

F020 4646

EOR

F002 37

F010 4.9

F004 5

F005 RE

F006 Falk-Petersen, I-B.,(1979)

- ** Toxic effects of aqueous extracts of Ekofisk crude oil, crude oil
- * fractions and commercial oil products on the development of sea urchin
- * eggs
- ** Sarsia, vol.64, pp. 161-169.

F007 Falk-Petersen, I-B.,(1979)

- ** Toxic effects of aqueous extracts of Ekofisk crude oil, crude oil
- * fractions and commercial oil products on the development of sea urchin
- * eggs
- ** Sarsia, vol.64, pp. 161-169.

F008 IUC4

F009 13-11-2003

F020 4642

EOR

F002 37

F010 4.9

F004 5

F005 RM

F006 Falk-Petersen has studied the effects of the water-soluble fraction (WSF)

- * of Ekofisk crude oil on the development of the eggs of two sea urchin
- * species, Strongylocentrotus pallidus and S. droebachiensis. The WSF was
- * prepared by shaking Ekof

F007 Falk-Petersen has studied the effects of the water-soluble fraction (WSF)

- * of Ekofisk crude oil on the development of the eggs of two sea urchin
- * species, Strongylocentrotus pallidus and S. droebachiensis. The WSF was
- * prepared by shaking Ekofisk crude oil and sea water in the ratio 1:9 for
- * 5 minutes, allowing to separate for 20 hours, and using the aqueous phase
- * for the studies.
- ** Fertilized eggs were exposed to dilutions of the WSF for up to 9 days at
- * 3 to 5 °C. Embryos and larvae were examined regularly by scanning and
- * transmission electron microscopy after fixing in 2% OsO4 in sea water.
- ** Concentrations of 30% WSF, corresponding to 13 ppm dissolved oil, did not
- * impair either development or the ultrastructure of the stages from egg to
- * pluteus. However, significant effects on development were found at both
- * 40%
- ** $\,$ WSF (17 ppm oil) and 50% WSF (21 ppm oil). At these levels, the larvae
- * filled with degenerating cells, and differentiation of the intestine and
- * skeletal growth were inhibited compared with the control larvae.

F020 4641

EOR

F002 37

F010 4.9

F004 5

F005 RM

F006 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- * reliability not assigned

F007 These data are also cited in the European Chemicals Bureau IUCLID for CAS

- * 8002-05-9. These data are ranked by API crude oil task group as '4',
- * reliability not assigned

F020 4643

EOR

F002 37

F010 4.9

F004 5

F005 SO

F006 CONCAWE Bruxelles

**

F007 CONCAWE Bruxelles

**

F008 IUC4

F009 09-10-2003

F020 4647

EOR

F002 37

F010 5.1.1

F004 1

F005 ME

F006 Groups of five male and five female Sprague-Dawley rats were dosed once

- * by oral gavage with the test material at a dose level of 5 g/kg.
- ** The animals were observed frequently on the day of treatment and daily
- * thereafter for 14 days.
- ** The anim

F007 Groups of five male and five female Sprague-Dawley rats were dosed once

- * by oral gavage with the test material at a dose level of 5 g/kg.
- ** The animals were observed frequently on the day of treatment and daily
- thereafter for 14 days.
- ** The animals were weighed on the day of treatment and again 7 and 14 days
- * later.

F020 4648

EOR

F002 37

F010 5.1.1

F004 1

F005 RE

F006 Mobil (1984)

- ** The acute oral toxicity of beryl crude in albino rats
- ** MEHSL Study No. 40951
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1984)

- ** The acute oral toxicity of beryl crude in albino rats
- ** MEHSL Study No. 40951
- ** Mobil Environmental and Health Science Laboratory

F020 4649

EOR

F002 37

F010 5.1.1

F004 1

F005 RL

F006 Not clear whether this study was carried out to GLP and few

- ** experimental data given.
- ** Nevertheless, the study is sufficient to demonstrate an LD50 of greater
- * than 5 g/kg.

F007 Not clear whether this study was carried out to GLP and few

- ** experimental data given.
- ** Nevertheless, the study is sufficient to demonstrate an LD50 of greater
- * than 5 g/kg.

F008 IUC4

F020 4650

EOR

F002 37

F010 5.1.1

F004 1

F005 RS

F006 There were no deaths following treatment.

- ** During the first week post dosing lacrimation and a
- ** discharge covering the perineum (genital area) were
- ** observed.
- ** At termination of the study all animals appeared healthy and
- ** had gained weight.
- ** The

F007 There were no deaths following treatment.

- ** During the first week post dosing lacrimation and a
- ** discharge covering the perineum (genital area) were
- ** observed.
- ** At termination of the study all animals appeared healthy and
- ** had gained weight.
- ** The oral LD50 was judged to be greater than 5 g/kg.

F008 IUC4

F020 4651

EOR

F002 37

F010 5.1.1

F004 3

F005 RE

F006 Mobil (1984)

- ** The acute oral toxicity of Arab light crude in Albino rats
- ** Study No. 40961
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1984)

- ** The acute oral toxicity of Arab light crude in Albino rats
- ** Study No. 40961
- ** Mobil Environmental and Health Science Laboratory

F020 4652

EOR

F002 37

F010 5.1.1

F004 3

F005 RE

F006 Mobil (1984)

** The acute oral toxicity of MCSL crude (Midcontinent) in

- ** albino rats
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1984)

- ** The acute oral toxicity of MCSL crude (Midcontinent) in
- ** albino rats
- ** Mobil Environmental and Health Science Laboratory

F020 4653

EOR

F002 37

F010 5.1.1

F004 3

F005 RE

F006 Mobil (1990)

- ** Consolidated acute test report on Lost Hills light crude
- ** Study Nos. 63830, 63831, 63832, 63833
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1990)

- ** Consolidated acute test report on Lost Hills light crude
- ** Study Nos. 63830, 63831, 63832, 63833
- ** Mobil Environmental and Health Science Laboratory

F020 4654

EOR

F002 37

F010 5.1.1

F0043

F005 RE

F006 Mobil (1997)

- ** Toxicological and compositional data on petroleum refinery heavy streams
- ** Mobil Environmental and Health Sciences Laboratory

F007 Mobil (1997)

- ** Toxicological and compositional data on petroleum refinery heavy streams
- ** Mobil Environmental and Health Sciences Laboratory

F020 4657

EOR

F002 37

F010 5.1.1

F004 3

F005 RE

F006 Smith, L. H., Haschek, W. M. and Witschi, H. (1981)

- ** Acute toxicity of selected crude and refined shale oil and
- ** petroleum-derived substances.
- ** In Greist, W. H., et al (Eds)
- ** Health Investigation of Oil Shale develpment pp 141-160.
- ** Ann Arbor Sc

F007 Smith, L. H., Haschek, W. M. and Witschi, H. (1981)

- ** Acute toxicity of selected crude and refined shale oil and
- ** petroleum-derived substances.
- ** In Greist, W. H., et al (Eds)
- ** Health Investigation of Oil Shale develpment pp 141-160.
- ** Ann Arbor Science, Mich.

F020 4655

EOR

F002 37

F0043 F005 RS F006 The results of other acute toxicity studies which have been reported are as follows: LD50 Clinical signs Sample Light crudes Lost Hills Light (Mobil consolidated report) Soft stool >5 g/kg Urogenital discharge Decreased feca F007 The results of other acute toxicity studies which have been reported are as follows: Sample LD50 Clinical signs Light crudes Lost Hills Light (Mobil consolidated report) >5 g/kg Soft stool ** Urogenital discharge Decreased fecal output Anal discharge MCSL Crude (midcontinent) Mobil study No. 40971 >5 g/kg Decreased activity Hunching Discharge/perineal staining Arab Light (Mobil study No. 40961) Discharge/perineal staining >5 g/kg Belridge heavy (Mobil summarized data) >5 g/kg Mild gastrointestinal effects Smith et al (1981) also demonstrated that three different crude oils (Crude type not specified) were non-toxic in male mice as follows: Wilmington Crude oil >16 g/kg Recluse crude oil >16 g/kg Mixed petroleum crudes >10 g/kg F020 4656 **EOR** F002 37 F010 5.1.3 F004 1 F005 ME

 ${\sf F006}$ The skin was clipped from the trunks of three male and three female ${\sf New}$

- * Zealand White rabbits prior to treatment with test material. The skin of
- * the backs of three animals (2 male, 1 female) was abraded and the skin of
- * the remaining animals

F010 5.1.1

F007 The skin was clipped from the trunks of three male and three female New

- * Zealand White rabbits prior to treatment with test material. The skin of
- * the backs of three animals (2 male, 1 female) was abraded and the skin of

- * the remaining animals was left intact. The liquid, undiluted test
- * material was then applied as a single dose of 2 g/kg to the shorn back of
- * all six animals.
- ** The test site was covered with a gauze and an occlusive
- ** wrap. The animals were also fitted with Elizabethan collars
- ** to prevent chewing of the occlusive covering and ingestion
- ** of test material.
- ** 24 hours after application of the test material the occlusive dressing
- * was removed and any surplus test material was removed from the skin by
- * gently wiping with cotton moistened with physiological saline.
- ** The animals were observed frequently on the day of treatment and daily
- * thereafter for 14 days.

- ** Two hours after wiping the residual material from the skin
- ** (26 hours after treatment), the areas of application were
- ** assessed for skin irritation. Further evaluations for skin
- ** irritation were also made on the 3rd and 7th days following
- ** treatment.

F008 IUC4

F020 4658

EOR

F002 37

F010 5.1.3

F004 1

F005 RE

F006 Mobil (1984)

- ** The acute dermal toxicity of Beryl crude in albino rabbits
- ** Study No. 40952
- ** Mobil Environmental and Health Sciences laboratory

F007 Mobil (1984)

- ** The acute dermal toxicity of Beryl crude in albino rabbits
- ** Study No. 40952
- ** Mobil Environmental and Health Sciences laboratory

F008 IUC4

F020 4659

EOR

F002 37

F010 5.1.3

F004 1

F005 RL

F006 Not clear whether this study was carried out to GLP and few

** experimental data given.

F007 Not clear whether this study was carried out to GLP and few

** experimental data given.

F008 IUC4

F020 4660

EOR

F002 37

F010 5.1.3

F004 1

F005 RS

F006 There were no deaths in this study.

** A few animals had soft stool/diarrhea during the observation period,

- * however, all animals were normal at study termination.
- ** At the end of the study five of the six animals had gained
- ** weight and one had lo

F007 There were no deaths in this study.

- ** A few animals had soft stool/diarrhea during the observation period,
- * however, all animals were normal at study termination.
- ** At the end of the study five of the six animals had gained
- ** weight and one had lost a small amount of weight. [No actual body weight
- data are provided in the report].

**

- ** There was no evidence of systemic toxicity during the study
- ** although there was some skin reaction at the site of
- ** application.
- ** Following 24 hours of skin contact, varying degrees (slight, moderate)
- * irritation were observed at the 26 and 72 hour readings. At the end of
- * the first week, the skin response was barely perceptible or absent.
- ** The dermal LD50 was judged to be greater than 2 g/kg

F008 IUC4

F020 4661

EOR

F002 37

F010 5.1.3

F004 2

F005 RE

F006 Mobil (1984)

- ** The acute dermal toxicity of Arab light crude in albino
- ** rabbits
- ** Study No. 40962
- ** Mobil Environmental and Health Sciences laboratory

F007 Mobil (1984)

- ** The acute dermal toxicity of Arab light crude in albino
- ** rabbits
- ** Study No. 40962
- ** Mobil Environmental and Health Sciences laboratory

F020 4663

EOR

F002 37

F010 5.1.3

F004 2

F005 RE

F006 Mobil (1984)

- ** The acute dermal toxicity of MCSL crude (Midcontinent) in
- ** albino rabbits
- ** Study No. 40972
- ** Mobil Environmental and Health Sciences laboratory

F007 Mobil (1984)

- ** The acute dermal toxicity of MCSL crude (Midcontinent) in
- ** albino rabbits
- ** Study No. 40972
- ** Mobil Environmental and Health Sciences laboratory

F020 4662

EOR

F002 37

```
F010 5.1.3
F004 2
F005 RE
F006 Mobil (1990)
** Consolidated acute test report on Lost Hills light crude
** Study Nos. 63830, 63831, 63832, 63833
** Mobil Environmental and Health Science Laboratory
F007 Mobil (1990)
** Consolidated acute test report on Lost Hills light crude
** Study Nos. 63830, 63831, 63832, 63833
** Mobil Environmental and Health Science Laboratory
F020 4664
EOR
F002 37
F010 5.1.3
F004 2
F005 RE
F006 Mobil (1997)
** Toxicological and compositional data on petroleum refinery heavy streams
** Mobil Environmental and Health Sciences Laboratory
F007 Mobil (1997)
** Toxicological and compositional data on petroleum refinery heavy streams
** Mobil Environmental and Health Sciences Laboratory
F020 4666
EOR
F002 37
F010 5.1.3
F004 2
F005 RS
F006 Additionally, acute dermal LD50s have also been reported for three other
   light crude oils.
   The results are as follows:
   Sample
                               LD50
                                                               Clinical signs
   Lost Hills Light (Mobil Study No. 63831)
                               >2 g/kg
                                                               Soft stool
                                                               Decreased food consump
F007 Additionally, acute dermal LD50s have also been reported for three other
   light crude oils.
   The results are as follows:
   Sample
                               LD50
                                                               Clinical signs
   Lost Hills Light (Mobil Study No. 63831)
                               >2 g/kg
                                                               Soft stool
                                                               Decreased food consumption
                                                               Decreased fecal output
   MCSL Crude (midcontinent) Mobil study No. 40972
                               >2 g/kg
                                                               Diarrhea
**
                                                               Nasal discharge
   Arab Light (Mobil study No. 40962)
```

>2 g/kg

No signs of toxicity

```
Belridge Heavy (Mobil summarized data)
                                >2 g/kg
                                                                Mild gastrointestinal
                                effects
F020 4665
EOR
F002 37
F010 5.2.1
F004 1
F005 CL
F006 The classification according to various methods/criteria are summarized
   as follows:
   Guideline
                                                Score
                                                                                Rating
   DOT Corrosion
                                                Negative
                                                                Non-corrosive
   EEC (4hr occluded)
                                Erythema 1.6
                                                                Non-irritant
                                                Edema 1.3
   OSHA PII (4 h occl.)
                                2.8
                                                                Non-i
F007 The classification according to various methods/criteria are summarized
   as follows:
   Guideline
                                                Score
                                                                                Rating
   DOT Corrosion
                                                Negative
                                                                Non-corrosive
   EEC (4hr occluded)
                                Erythema 1.6
                                                                Non-irritant
                                                Edema 1.3
** OSHA PII (4 h occl.)
                                2.8
                                                                Non-irritant
** FHSA PII (24 h occl.)
                                                                Non-irritant
F008 IUC4
F020 4667
EOR
F002 37
F010 5.2.1
F004 1
F005 ME
F006 Prior to test the hair was clipped from the backs of six New Zealand
   White rabbits (sex not specified). Three one inch square sites
   (anterior, mid-dorsal and posterior) on the right side of each animal
   were lightly abraded. Three
   similarly
F007 Prior to test the hair was clipped from the backs of six New Zealand
   White rabbits (sex not specified). Three one inch square sites
```

(anterior, mid-dorsal and posterior) on the right side of each animal

similarly orientated sites were left intact on the left side of the

* animal, thus providing six test sites on each rabbit.
** 0.5 ml of test material was applied to each test site. The
** material applied to the anterior and mid-dorsal sites was
** covered with a Webril patch and these were occluded. The

were lightly abraded. Three

- ** dorsal sites remained open. The rabbits were fitted with
- ** collars to prevent ingestion of the test material an chewing of the
- occluded patches.

- ** Four hours after treatment, the anterior sites were
- ** unwrapped. The intact site was immediately examined for
- ** evidence of corrosive effects and both sites were then gently wiped with
- * a gauze. Thirty minutes later, the skin was evaluated for irritation. The
- * sites were evaluated again 24 hours after patch removal. 48 hours after
- * application, the 4 hour occluded intact site was re assessed for
- * corrosion. Both sites were scored for irritation 4 hours later. The 4
- * hour occluded sites were again re-evaluated for irritation on day 3.

**

- ** 24 hours after treatment, the two mid-dorsal sites were
- ** unwrapped and along with the posterior uncovered sites were
- ** wiped with cotton. The four sites were evaluated for
- ** irritation 2 hours later, again approximately 72 hours later and finally
- * on the 7th day after treatment.

F008 IUC4

F020 4668

EOR

F002 37

F010 5.2.1

F004 1

F005 RE

F006 Mobil (1990)

- ** Consolidated acute test report on Lost Hills light crude
- ** Study Nos. 63830, 63831, 63832, 63833
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1990)

- ** Consolidated acute test report on Lost Hills light crude
- ** Study Nos. 63830, 63831, 63832, 63833
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F009 03-10-2002

F020 4669

EOR

F002 37

F010 5.2.1

F004 1

F005 RS

F006 4 Hour occluded sites (DOT, OECD methods)

**

- ** Mean values (24, 48 & 72 hours) for erythema and edema at
- ** the intact sites were 1.69 and 1.3 respectively.
- ** $\,$ The initial response $\,$ of the skin to the test material was
- ** slight, with little difference

F007 4 Hour occluded sites (DOT, OECD methods)

**

- ** Mean values (24, 48 & 72 hours) for erythema and edema at
- ** the intact sites were 1.69 and 1.3 respectively.
- ** The initial response of the skin to the test material was
- ** slight, with little difference in response between intact or

abraded sites. Actual scores were: Intact Abraded Erythema Edema Erythema Edema 4.5 hrs 1.0 1.2 1.0 1.3 28 hrs 1.2 1.0 1.0 1.0 52 hrs 1.7 1.3 1.7 1.3 76 hrs 1.7 1.8 1.5 1.8 7 days 8.0 0.7 0.5 0.7 10 days 0 0.3 0 0 24 Hour occluded sites (FHSA method) Actual scores were: ** Intact Abraded Edema Erythema Erythema Edema ** 26 hrs 1.7 1.7 1.7 1.7 72 hrs 2.3 2.2 1.5 1.7 0.7 7 days 1.2 8.0 0.8 10 days 0.2 0 0.2 0.2 Non-occluded sites Actual scores were: Intact Abraded Erythema Edema Erythema Edema 26 hrs 1.7 1.2 1.8 1.3 72 hrs 2.8 1.5 2.7 1.3 7 days 1.3 0.8 1.3 8.0 ** 10 days 0.3 0 0.2 0 F020 4670

F008 IUC4

EOR

F002 37

F010 5.2.1

F004 2

F005 ME

F006 Prior to test the hair was clipped from the backs of six New Zealand

- White rabbits (sex not specified). Three one inch square sites
- (anterior, mid-dorsal and posterior) on the right side of each animal
- were lightly abraded. Three
- similarly

F007 Prior to test the hair was clipped from the backs of six New Zealand

- White rabbits (sex not specified). Three one inch square sites
- (anterior, mid-dorsal and posterior) on the right side of each animal
- were lightly abraded. Three
- similarly orientated sites were left intact on the left side of the

- * animal, thus providing six test sites on each rabbit.
- ** 0.5 ml of test material was applied to each test site. The
- ** material applied to the anterior and mid-dorsal sites was
- ** covered with a Webril patch and these were occluded. The
- ** dorsal sites remained open. The rabbits were fitted with
- ** collars to prevent ingestion of the test material and chewing of the
- * occluded patches.

- ** Four hours after treatment, the anterior sites were
- ** unwrapped. The intact site was immediately examined for
- ** evidence of corrosive effects and both sites were then gently wiped with
- * a gauze. Thirty minutes later, the skin was evaluated for irritation. The
- * sites were evaluated again 24 hours after patch removal. 48 hours after
- * application, the 4 hour occluded intact site was re assessed for
- * corrosion. Both sites were scored for irritation 4 hours later. The 4
- * hour occluded sites were again re-evaluated for irritation on day 3.

*

- ** 24 hours after treatment, the two mid-dorsal sites were
- ** unwrapped and along with the posterior uncovered sites were
- ** wiped with cotton. The four sites were evaluated for
- ** irritation 2 hours later, again approximately 72 hours later and finally
- * on the 7th day after treatment.

F008 IUC4

F020 4671

EOR

F002 37

F010 5.2.1

F004 2

F005 RE

F006 Mobil (1985)

- ** Skin irritation by Arab light crude after single
- ** applications, occluded and non-occluded, on albino rabbits
- ** Study No. 40964
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1985)

- ** Skin irritation by Arab light crude after single
- ** applications, occluded and non-occluded, on albino rabbits
- ** Study No. 40964
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4672

EOR

F002 37

F010 5.2.1

F004 2

F005 RL

F006 A textual description of the results only was available. No

** actual data were provided in the report.

F007 A textual description of the results only was available. No

** actual data were provided in the report.

F008 IUC4

F020 4673

```
EOR
F002 37
F010 5.2.1
F004 2
F005 RS
F006 4 Hour Occluded (OECD Method, DOT)
** Mean values (24, 48 & 72 hours) for erythema and edema at
** the intact sites were 0.9 and 0.1 respectively.
   The initial response of the skin to the test material was
   slight. Within 72 hours after treatment
F007 4 Hour Occluded (OECD Method, DOT)
** Mean values (24, 48 & 72 hours) for erythema and edema at
** the intact sites were 0.9 and 0.1 respectively.
** The initial response of the skin to the test material was
** slight. Within 72 hours after treatment, most sites had
   recovered, with little difference in response between intact or abraded
   sites.
   24 Hour Occluded (FHSA method)
** The day following application of the test material a
  moderate erythema was observed. This subsided during the
   subsequent days such that there was only barely perceptible
   irritation at the end of the first week. Abrasion had no
   effect on either the initial response or the recovery.
   Non Occluded
** Uncovered treated sites had moderate erythema 24 hours after application
   of the test material. This response diminished and was virtually absent
   after one week.
** Abrasion had no effect on either the initial response or the recovery.
F008 IUC4
F020 4674
EOR
F002 37
F010 5.2.1
F004 3
F005 RE
F006 Mobil (1997)
** Toxicological and compositional data on petroleum refinery heavy streams
** Mobil Environmental and Health Sciences Laboratory
F007 Mobil (1997)
** Toxicological and compositional data on petroleum refinery heavy streams
** Mobil Environmental and Health Sciences Laboratory
F020 4676
EOR
F002 37
F010 5.2.1
F004 3
F005 RL
```

F006 Data only available in tabular form, but studies were part of a series of

* studies using same methods, all of which were reliability 1.

F007 Data only available in tabular form, but studies were part of a series of

* studies using same methods, all of which were reliability 1.

F020 4677

EOR

F002 37

F010 5.2.1

F0043

F005 RS

F006 Summarized data for Belridge Heavy crude oil are:

**

** Guideline Score Rating

*

** DOT Corrosion Negative Non-corrosive

** EEC (4hr occluded) Erythema 0.6 Non-irritant

* Edema 0.8

**

** OSHA PII (4 h occl.) 1.4 Non-irritant

** FHSA PII (24 h occl.) 2.1

F007 Summarized data for Belridge Heavy crude oil are:

**

** Guideline Score Rating

**

** DOT Corrosion
 ** EEC (4hr occluded)
 ** Erythema 0.6
 Non-corrosive
 Non-irritant

* Edema 0.8

**

** OSHA PII (4 h occl.) 1.4 Non-irritant

** FHSA PII (24 h occl.) 2.1 Non-irritant

F020 4675

EOR

F002 37

F010 5.2.2

F004 1

F005 ME

F006 0.1 ml of undiluted test material was instilled into one eye

- ** of each of six New Zealand White rabbits.
- ** The eyes were not washed and were evaluated for irritation
- ** at 1, 24, 48 and 72 hours after treatment. Fluorescein was
- ** used to aid the eva

F007 0.1 ml of undiluted test material was instilled into one eye

- ** of each of six New Zealand White rabbits.
- ** The eyes were not washed and were evaluated for irritation
- $^{\star\star}~$ at 1, 24, 48 and 72 hours after treatment. Fluorescein was
- ** used to aid the evaluation of the 72 hour reading.
- ** Treated eyes that stained positively with fluorescein were
- ** examined again 7 and 14 days after treatment.

F008 IUC4

F020 4678

EOR

F002 37

F010 5.2.2

F004 1

F005 RE

F006 Mobil (1985)

- ** Primary eye irritation of Beryl crude in albino rabbits
- ** Study No. 40953
- ** Mobil Environmental and Health Sciences Laboratory

F007 Mobil (1985)

- ** Primary eye irritation of Beryl crude in albino rabbits
- ** Study No. 40953
- ** Mobil Environmental and Health Sciences Laboratory

F008 IUC4

F009 03-10-2002

F020 4679

EOR

F002 37

F010 5.2.2

F004 1

F005 RL

F006 The only reservation is that it is not clear whether this

- ** study was carried out according to GLP.
- ** Otherwise the study and reporting are sound.

F007 The only reservation is that it is not clear whether this

- ** study was carried out according to GLP.
- ** Otherwise the study and reporting are sound.

F008 IUC4

F020 4680

EOR

F002 37

F010 5.2.2

F004 1

F005 RS

F006 The mean irritation scores at each of the observation times

** were:

**

**		1 hr	24 hr	48 hr	72 hr
**	Cornea	0	0	0	0
**	Iris	0	0	0	0
**	Conjunctivae	4.0	1.7	1.3	1.0

**

- ** The test material was judged to be non-irritating to the
- ** rabbit eye.

F007 The mean irritation scores at each of the observation times

** were:

**

**		1 hr	24 hr	48 hr	72 hr
**	Cornea	0	0	0	0
**	Iris	0	0	0	0
**	Conjunctivae	4.0	1.7	1.3	1.0

k*

- ** The test material was judged to be non-irritating to the
- ** rabbit eye.

F008 IUC4

F020 4681

EOR F002 37 F010 5.2.2 F004 2 F005 RE F006 Mobil (1985) ** Primary eye irritation of Arab light crude in albino rabbits ** Study No. 40963 ** Mobil Environmental and Health Science Laboratory F007 Mobil (1985) ** Primary eye irritation of Arab light crude in albino rabbits ** Study No. 40963 ** Mobil Environmental and Health Science Laboratory F008 IUC4 F020 4682 **EOR** F002 37 F010 5.2.2 F004 2 F005 RE F006 Mobil (1985) ** Primary eye irritation of MSCL Crude (Midcontinent) in ** albino rabbits. ** Study No. 40973 ** Mobil Environmental and Health Sciences Laboratory F007 Mobil (1985) ** Primary eye irritation of MSCL Crude (Midcontinent) in ** albino rabbits. ** Study No. 40973 ** Mobil Environmental and Health Sciences Laboratory F008 IUC4 F020 4683 EOR F002 37 F010 5.2.2 F004 2 F005 RE F006 Mobil (1990) ** Consolidated acute test report on Lost Hills light crude ** Study Nos. 63830, 63831, 63832, 63833 ** Mobil Environmental and Health Science Laboratory F007 Mobil (1990) ** Consolidated acute test report on Lost Hills light crude ** Study Nos. 63830, 63831, 63832, 63833

** Mobil Environmental and Health Science Laboratory

F008 IUC4 F009 03-10-2002

F020 4684

EOR

F002 37

F010 5.2.2

F004 2

F005 RE

F006 Mobil (1997)

- ** Toxicological and compositional data on petroleum refinery heavy streams
- ** Mobil Environmental and Health Sciences Laboratory

F007 Mobil (1997)

- ** Toxicological and compositional data on petroleum refinery heavy streams
- ** Mobil Environmental and Health Sciences Laboratory

F020 4686

EOR

F002 37

F010 5.2.2

F004 2

F005 RS

F006 Eye irritation studies have been reported for three other

- ** samples of light crude oil.
- ** There were no effects on either the iris or cornea. The only effects
- * recorded were on the conjunctivae.
- ** The mean irritation scores (conjunctivae only) are

F007 Eye irritation studies have been reported for three other

- ** samples of light crude oil.
- ** There were no effects on either the iris or cornea. The only effects
- * recorded were on the conjunctivae.
- ** The mean irritation scores (conjunctivae only) are as
- ** follows:

k*

**	Crude type		Result			Ref
**		1 hr	24 hr	48 hr	72 hr	
**	MCSL (Midcontinent)					
**		2.3	0.3	0	0.7	Mobil 40973
**						
**	Lost Hills Light					
**		8.0	3.7	2.7	1.7	Mobil 63832
**						
**	Arab Light					
**		5.3	1.3	0.7	0.3	Mobil 40963
**	Belridge Heavy		0.9	0.8	0.9	Mobil 1997

F020 4685

EOR

F002 37

F010 5.3

F004 1

F005 ME

F006 Concentrations of test material used in this sensitization

** study were determined in a pre screening study.

**

- ** Induction phase
- ** 0.4 ml of a 15% w/w concentration of test material in
- ** mineral oil was applied using a Hill Top Chamber with a 25
- ** mm

F007 Concentrations of test material used in this sensitization

** study were determined in a pre screening study.

..

- ** Induction phase
- ** 0.4 ml of a 15% w/w concentration of test material in

- ** mineral oil was applied using a Hill Top Chamber with a 25
- ** mm Webril swatch to the shorn backs of 10 male and 10 female Guinea pigs.
- * The patch was occluded for six hours.
- ** The patches were applied to the same site once per week for
- ** 3 weeks. After each 6-hour exposure period, he patches were
- ** removed and the skin wiped with gauze moistened with mineral oil.
- ** The positive control material (DNCB) was applied at a
- ** concentration of 0.05% w/w in 70% ethanol to 5 male and 5
- ** female Guinea pigs. Treatment of the positive control
- ** animals was the same as for the test animals, except that
- ** the skin was wiped with saline after patch removal.

*

- ** Challenge
- ** Challenge patch application was performed 14 days after the
- ** last induction dose had been applied.
- ** Dual challenge patches were applied to fresh application
- ** sites of previously shorn skin of the animals. The test
- ** material was applied at concentrations of 10 and 15% w/w in
- ** mineral oil. The patches were then occluded for 6 hours.
- ** On the day following challenge patch application, the skin
- ** was depilated and 2 hours later and scored for signs of
- ** sensitization. The sites were examined after a further 48
- ** hours but this time without depilation.
- ** Naive and positive control animals were challenged with DNCB at a
- * concentration of 0.05% in acetone.

F008 IUC4

F020 4687

EOR

F002 37

F010 5.3

F004 1

F005 RE

F006 Mobil (1991)

- ** Delayed contact hypersensitivity study in Guinea pigs
- ** (Buehler sensitization test) of Lost Hills light crude oil
- ** Study No. 63841
- ** Environmental and Health Sciences Laboratory

F007 Mobil (1991)

- ** Delayed contact hypersensitivity study in Guinea pigs
- ** (Buehler sensitization test) of Lost Hills light crude oil
- ** Study No. 63841
- ** Environmental and Health Sciences Laboratory

F008 IUC4

F020 4688

EOR

F002 37

F010 5.3

F004 1

F005 RS

F006 The results were as follows:

** No. Mean erythema

responding score

** Test material (10% concentration)

**	24 hrs induced	3/19	0.2
**	24 hrs control	0/10	0
**			
**	48 hrs induced	3/19	0.3
**	48 hrs control	2/10	0.2
**			

** Test material (15% concentrat

F007 The results were as follows:

**		No.		Mean erythema
**		responding	score	
**	Test material (10% concentration)			
**	24 hrs induced	3/19		0.2
**	24 hrs control	0/10		0
**				
**	48 hrs induced	3/19		0.3
**	48 hrs control	2/10		0.2
**				
**	Test material (15% concentration)			
**	24 hrs induced	2/19		0.2
**	24 hrs control	2/10		0.2
**				
**	48 hrs induced	1/19		0.1
**	48 hrs control	1/10		0.1
**				
**	DNCB positive control			
**	24 hrs induced	5/5		3.4
**	24 hrs control	0/5		0
**				
**	48 hrs induced	5/5		2.6
**	48 hrs control	0/5		0
**				

- ** Although two test animals responded, the test material was
- ** judged to be non-sensitizing, since the test criterion
- ** required an mean erythema score of +2 for a positive
- ** response.

F008 IUC4

F020 4689

EOR

F002 37

F010 5.3

F004 2

F005 RE

F006 Mobil (1991)

- ** Delayed contact hypersensitivity study in Guinea pigs
- ** (Buehler sensitization test) of Belridge Heavy crude oil
- ** Study No. 63853
- ** Environmental and Health Sciences Laboratory

F007 Mobil (1991)

- ** Delayed contact hypersensitivity study in Guinea pigs
- ** (Buehler sensitization test) of Belridge Heavy crude oil
- ** Study No. 63853
- ** Environmental and Health Sciences Laboratory

F020 4691

F004 2 F005 RS F006 A similar Buehler test of Belridge heavy crude oil was also F007 A similar Buehler test of Belridge heavy crude oil was also ** negative. F020 4690 **EOR** F002 37 F010 5.4 F004 1 F005 ME F006 Two separate but identical studies are reported, one for ** each of two crude oils (Crude I and Crude II). The methods used were identical for both studies. Undiluted test material was applied to the shorn unoccluded ** skin of groups of ten mal F007 Two separate but identical studies are reported, one for each of two crude oils (Crude I and Crude II). The methods used were identical for both studies. Undiluted test material was applied to the shorn unoccluded skin of groups of ten male and ten female Sprague-Dawley ** rats at doses of 30, 125 and 500 mg/kg/day. Application was once daily, five times each week for 13 weeks. Groups of ten rats of each sex served as untreated controls. ** Each animal was fitted with an Elizabethan collar to prevent ingestion of the applied test material. At the end of each week residual test material was wiped ** from the backs of the animals. ** Animals were observed regularly for clinical signs of toxicity and body weights were recorded weekly. The animals were sacrificed during week 14 of the study ** after fasting overnight. All animals were necropsied and blood samples were taken for a range of hematological and serum chemistry determinations. A range of organs were weighed and tissues examined histologically. An additional two groups of ten males were treated at a dose level of 0 and 500 mg/kg/day for an evaluation of male reproductive health.

For these animals testes weight and cauda epididymis weights were recorded. Additionally, the number of sperm and % normal sperm in the cauda were recorded as well as the number of spermatids in the testis.

F008 IUC4 F020 4692 EOR

EOR F002 37 F010 5.3

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F002 37
F010 5.4
F004 1
F005 RE
F006 Feuston, M. H., Mackerer, C. M., Schreiner, C. A. and
** Hamilton, C. E. (1997)
** Systemic toxicity of dermally applied crude oils in rats
** J. Tox. Env. Health, Vol 51, pp 387-399
F007 Feuston, M. H., Mackerer, C. M., Schreiner, C. A. and
** Hamilton, C. E. (1997)
** Systemic toxicity of dermally applied crude oils in rats
** J. Tox. Env. Health, Vol 51, pp 387-399
F008 IUC4
F020 4693
EOR
F002 37
F010 5.4
F004 1
F005 RE
F006 Mobil (1992)
** Thirteen-week dermal administration of Lost Hills Light to
** Study No. 63834
** Environmental Health and Sciences Laboratory
F007 Mobil (1992)
** Thirteen-week dermal administration of Lost Hills Light to
** rats
** Study No. 63834
** Environmental Health and Sciences Laboratory
F008 IUC4
F020 4694
EOR
F002 37
F010 5.4
F004 1
F005 RS
F006 No animals died in either of the studies and there were no
** clinical signs of systemic toxicity attributable to either
** crude oil. Both crude oils caused the same minimal skin
   irritation (flaking) at the exposure site.
** Body weights
** Apart fro
F007 No animals died in either of the studies and there were no
** clinical signs of systemic toxicity attributable to either
** crude oil. Both crude oils caused the same minimal skin
** irritation (flaking) at the exposure site.
** Body weights
** Apart from the 500 mg/kg/day group for Crude II that gained
```

** less weight than the controls (217g compared to 247g over
** the course of the study) all other body weight measurements

** were similar to the respective controls.

```
**
```

- ** Hematology
- ** Significant changes relative to their respective controls
- ** only occurred in the highest dose group animals (500
- ** mg/kg/day). The % increases (+) or decreases (-) that
- ** occurred in these dose groups are shown in the following
- ** table. [Changes are shown to the nearest whole number]

**

**	Parameter	Crude I			Crude II	
**		Males	Females		Males	Females
**						
**	RBC	-6%	NSD		-8%	-7%
**	Hemoglobin	-6%	NSD		-11%	-8%
**	Hematocrit	-5%	NSD		-9%	-8%
**	Platelets NSD	NSD		-19%	NSD	

**

* NSD No significant difference

**

- ** Serum chemistry
- ** Although more parameters were affected in the high dose
- ** Crude II females, there was no consistent pattern of change
- ** that could be attributed to the two crude oils. The
- ** differences %+ or %- are shown in the table below.

**

Dose group (mg/kg/day)

** Crude I		Crude I			Crude II	
**	30	125	500	30	125	500

** MALES

*

- ** Calcium -7%

 ** Glucose +13% +13%

 ** Urea nitrogen +53%
- ** Uric acid -27%

*

** FEMALES

*

	ALT Cholesterol		+41%	-18% +60%
**	Glucose +16%	+20%		
**	Potassium	-11%		-13%
**	Urea nitrogen			+31%
**	Uric acid			-27%

**

- ** Necropsy findings
- ** There were no treatment-related observations at necropsy.
- ** With the exception of the liver and thymus, there were no
- ** absolute or relative organ weight changes when compared to
- ** controls
- ** The organ weight differences that were observed were
- ** confined to the 125 and 500 mg/kg/day groups and are
- ** summarized below.

**

** Organ Crude I Crude II

**	wt			125	500	125	500	
**								
**	MALES							
**	Liver	(absolute)		+20%		+22%		
**	2.101	(relative)		+22%	+18%	+33%		
**	Thymus	(absolute)				-41%		
**								
**	FEMALES							
**								
**	Liver	(absolute)		+13%		+24%		
**	Thursus	(relative)		+12%		+31% -35%		
**	Thymus	(absolute) (relative)				-35% -27%		
**		(relative)				-21 /0		
**	Histopathol	ogy						
**		-						
**	Changes in	the skin included	d hyperplasia an	d an associated				
**			filtration. In gene					
**		-	evere in the anim					
**			emale animals e: e lowest dose le	•				
*		Crude II were aff		vei o/ io maie an	d 6/10 females			
**	oxpooda to	orado ii woro aii	00104.					
**	Other histor	oathological findi	ings were associ	ated with the				
**	bone marro	w, Liver, thymus	and thyroid alth	ough effects				
**	generally w	ere greater with	crude II than witl	n crude I.				
**	The finding	s are tabulated l	below					
**								
**	Crude I							
**	Bone marro	ıw.	No effects in ei	ther sex at any o	lose level			
**	Bono mano	•••	110 0110010 111 01	inor dox at any c	.000 10101			
**	Liver		Multifocal, mor	nonuclear cell in	filtration			
in	3 males and	2						
*	females at 5	500 mg/kg/day.						
**			Multifocal hepa	atocellular vacu	olation in 3			
ter *	nales at 500 only	mg/kg/day						
**	Offig							
**	Thymus		Atrophy in one	male and two fe	emales at 500			
**	,		mg/kg/day only					
**								
**	Thyroid		Hypertrophy ar	nd hyperplasia o	f follicular			
**			epithelium in s	ome males and	females at all			dose levels
**								
**	Crude II							
**	Bone marro	110/	Increased cells	larity in 2 males	at each			
do	se level (6	***	moreaseu celli	nanty in 2 maies	at Gaott			
*	•	hest dose group)			and focal necre	osis in 2 males in	the 50
*	3	3 1.		mg/kg/day grou	ıp.			
**			In the females	inaragand callula				

In the females increased cellularity was

observed in 9/10 animals

**

** Liver Hepatocellular vacuolation in one

male and one female in the

* 500 mg/kg/day group
** Mononuclear cell infiltration was also

observed but in only one

* male in the highest dose group

**

** Thymus Atrophy was observed in six males and 7

females in the 500

mg/g/day group.

**

** Thyroid Hypertrophy and hyperplasia was seen in a

few animals at all

dose levels.

**

- ** The spermatozoa/spermatid evaluations that were conducted on the separate
- * 500 mg/kg/day group of animals exposed to crude I did not reveal any
- * effects when compared to a control.

F008 IUC4

F020 4695

EOR

F002 37

F010 5.4

F004 1

F005 TS

F006 Two crude oils were examined in the paper by Feuston et al

** The characteristics of the crude oils was reported as:

**

**	Chemical class		Crude I	Crude II
**				
**	Nonaromatics		50.0	37.3
**	<3-Ring PAH		35.3	41.7
**	3- to 5- Ring PAH	10.2	15.7	
**	3- Ring PAH		5.9	

F007 Two crude oils were examined in the paper by Feuston et al

** The characteristics of the crude oils was reported as:

**

**	Chemical class		Crude I	Crude II
**				
**	Nonaromatics		50.0	37.3
**	<3-Ring PAH		35.3	41.7
**	3- to 5- Ring PAH	10.2	15.7	
**	3- Ring PAH		5.9	8.1
**	4- Ring PAH		2.2	4.0
**	5- Ring PAH		2.1	3.6
**	Sulfur-PAC		2.4	2.9
**	Nitrogen (N)-PAC	5.4	8.4	
**	Nonbasic N-PAC		3.8	5.9
**				
**	Total		103.3	106.0
**				

**

- ** From information available in the Mobil report of study No
- ** 63834 and other laboratory data, it is clear that Crude I is Lost Hills
- * Light Crude and Crude II is Belridge heavy crude oil.

F020 4696

EOR

F002 37

F010 5.4

F004 2

F005 ME

F006 Two pilot studies were conducted with groups of 5 male and 5 female mice.

- * In these studies Prudhoe Bay crude oil was
- ** administered by gavage at doses of 0, 5 and 10 ml/kg daily
- ** for five days. No other experimental details are given.
- ** However,

F007 Two pilot studies were conducted with groups of 5 male and 5 female mice.

- * In these studies Prudhoe Bay crude oil was
- ** administered by gavage at doses of 0, 5 and 10 ml/kg daily
- ** for five days. No other experimental details are given.
- ** However, the results led the author to conduct four further
- ** follow-up studies and these are described below.
- ** Experiment 1 (Three crudes, each at a single dose level)
- ** One of three crude oils or Bunker C oil was administered by
- ** gavage, daily at a single dose level of 10 ml/kg for 5 days
- ** to groups of 10 male mice. A further group of 10 male mice
- ** were intubated each day but no material was administered;
- ** these animals served as controls.
- **
- ** Experiment 2 (One crude, single dose level)
- ** Groups of 10 male mice were given either Prudhoe Bay crude
- ** oil or mineral oil USP or corn oil at a dose of
- ** 10 ml/kg/day for five days. A separate group of 10 controls were
- * intubated but no material was administered. This study was to compare the
- * effect of one crude oil with the effects of two oils expected to be
- * non-toxic.
- **
- ** Experiment 3 (One crude, five dose levels)
- ** Prudhoe bay crude oil was administered to 10 or 11 male mice at dose
- * levels of 2, 4, 8, 12 or 16 ml/kg/day. A further group of 10 male mice
- * wee sham treated and served as controls.
- **
- ** A fourth experiment was also carried out which studied the
- ** effect of crude oil exposure on vitamin E and
- ** selenium-deficient animals. This study is not summarized
- ** here.
- **
- ** In all experiments 1-3 the animals were weighed on the day
- ** they were first dosed and again prior to necropsy. The
- ** differences in weights were recorded as change in body
- ** weight during the studies. Necropsy was 24 hours after the
- ** 5th dose had been administered.

- ** At necropsy, blood samples were taken for determination of
- ** blood cell counts, packed cell volume (PCV), hemoglobin and red cell
- * indices (MCHC). Blood smears were stained with
- ** methylene blue to detect the presence of Heinz bodies in red cells.
- ** Liver was removed and weighed whereas the spleen and thymus
- ** were weighed after fixation. Portions of all major organs
- ** were fixed for subsequent histological examination.

**

- ** Data were analyzed by analysis of variance and regression
- ** procedures of the SAS programs (SAS Institute Inc., Cary,
- ** NC)

F008 IUC4

F020 4697

EOR

F002 37

F010 5.4

F004 2

F005 RE

F006 Leighton, F. A. (1990)

- ** The systemic toxicity of Prudhoe Bay crude and other
- ** petroleum oils to CD-1 mice.
- ** Arch. Environ. Contam. Toxicol Vol 19, pp257-262

F007 Leighton, F. A. (1990)

- ** The systemic toxicity of Prudhoe Bay crude and other
- ** petroleum oils to CD-1 mice.
- ** Arch. Environ. Contam. Toxicol Vol 19, pp257-262

F008 IUC4

F020 4698

EOR

F002 37

F010 5.4

F004 2

F005 RL

F006 These studies were probably not carried out according to GLP

- ** and the studies did not include measurement of all the
- ** parameters normally measured in repeat dose studies.
- ** Although the results are of limited value they do
- ** nevertheless add some

F007 These studies were probably not carried out according to GLP

- ** and the studies did not include measurement of all the
- ** parameters normally measured in repeat dose studies.
- ** Although the results are of limited value they do
- ** nevertheless add some information on the effect of the
- ** repeated exposure of mice to crude oil.

F008 IUC4

F020 4699

EOR

F002 37

F010 5.4

F004 2

F005 RS

F006 Although several studies were reported, the only studies

** summarized here are study A in which several crude oils are

- ** tested at a single dose level and study C in which PBCO is
- ** examined at five different dose levels.

**

** Neither information on

F007 Although several studies were reported, the only studies

- ** summarized here are study A in which several crude oils are
- ** tested at a single dose level and study C in which PBCO is
- ** examined at five different dose levels.

**

- ** Neither information on oils other than crude oil or on the
- ** effect of Vitamin E and selenium-depletion are included in
- ** this robust summary.

**

- ** In the pilot studies it was reported that the oils were
- ** distasteful to the mice and that after the first dose,
- ** administration of test material was difficult. Several mice
- ** from each group died from inhalation of oil that occurred
- ** during the oral dosing.
- ** The author stated that the pilot studies demonstrated a dose related
- * decrease (8-11%) in PCV, a reduction in body weight gain, a 74% increase
- * in liver weight per unit body weight and a 66% reduction in thymus
- * weight. No data are given but this was the reason given for the further
- * studies that were carried out.

**

- ** Although the studies carried out were separate, the author
- ** reported the results together and these are summarized
- * below.

**

- ** Hematology
- ** It was reported (but no data presented) that in experiment A none of the
- * oils tested resulted in significant changes in PCV, number of red blood
- * cells or whole blood hemoglobin.
- ** The following table summarizes the hematological results
- ** obtained with PBCO only.

**	Study	No of	Daily dose PBCO	PCV		MCHC
**		mice	(ml/kg/day)			(g/l)
**						
**	Α	10	0		0.43±0.02	336±22
**		8	10		0.42±0.04	328±10
**						
**	С	11	0		0.43±0.02	328±11
**		8	12		0.40±0.03	348±3*
**						

* *

p<0.05, analysis of variance with Tukey's multiple

range procedure

** Body and organ weights

- ** The organ and body weight changes are summarized in the
- ** following tables
- ** Study A comparison of three different crude oils

* Treatment group

** Control PBCO SLCO ALCO

```
** Group size
                                                                                         7
                             8
                                                           6
  Change in body wt (g)
   +1.74±0.88
                             -0.33±1.5
                                            -0.77±2.36*
                                                           -1.43±2.28*
** Liver wt (g)
** 2.15±0.2
                             3.03±0.54*
                                            2.73±0.5
                                                           2.66±0.43*
** Liver/body wt (g/g)
  0.07±0
                             0.11±0.01*
                                            0.10±0.01*
                                                           0.09±0.01*
** Spleen/body wt (g/g)
  3.52±0.61
                             2.46±0.24*
                                            2.49±0.29*
                                                           2.17±0.66*
** Thymus/body wt (g/g)
** 2.26±0.46
                             0.85±0.33*
                                            1.01±0.41*
                                                            1.12±0.46*
** * P<0.05
                             Analysis of variance with Tukey's multiple
range procedure
   Study C: PBCO at 5 dose levels
   Body wt
               Liver wt Organ/body wt (g/g)
   change (g)
                                              liver spleen thymus
                             (g)
** Control group (10 mice)
** +3.14±1.33 1.95±.18 .06±.01 3.9±.49 2.7±.29
** 2 ml/kg/day (10 mice)
  -0.88±2.57 2.32±.22 .09±0 2.65±.62 1.61±.49*
** 4 ml/kg/day (9 mice)
** -0.86±1.24 2.38±.33* .09±.01* 2.57±.23* 1.35±.32*
** 8 ml/kg/day (8 mice)
** -1.66±2.03* 2.58±.48* .1±.01* 2.31±.67* 1.03±.4*
** 12 ml/kg/day (9 mice)
** -1.66±1.71* 2.69±.27* .1±.01* 2.2±.44* .71±.22*
** 16 ml/kg/day (7 mice)
  -3.29±1.4* 2.15±.35 .1±.01* 2.26±.87* .65±.15*
** Coefficient of determination (R2)
** 0.49***
              0.08** 0.48*** 0.31*** 0.64***
                              P<0.05 Analysis of variance with Tukey's
multiple range procedure
                              P<0.03 Analysis of variance in regression
```

P<0.001 Analysis of variance in regression

. .

- ** Morphology
- ** There were no gross abnormalities at necropsy except a
- ** reduction in the size of thymus glands in those animals that had received
- * crude oil
- ** Liver, kidney, spleen, lung and thymus from groups of 5 mice that had
- * received 0, 5 or 10 ml PBCO for 5 days in the pilot study were examined
- * histologically. The thymus glands of mice in the 10 ml/kg/day group had
- * very thin cortices and reduced densities of lymphocytes in the remaining
- cortex compared to controls.
- ** Hepatocytes from these same animals had uniformly dense
- ** cytoplasm while control mouse hepatocytes had large areas of rarified
- * cytoplasm typical of normal glycogen-replete mouse livers. The histology
- * of the thymus glands of the 5
- ** ml/kg/day group was intermediate between the controls and
- ** the 10 ml/kg/day group.

**

- ** There were no histological lesions in the other tissues
- ** examined.

**

- ** Overall, the results demonstrated minor hematological
- ** change, liver enlargement and atrophy of spleen and thymus.

F008 IUC4

F020 4700

EOR

F002 37

F010 5.4

F004 2

F005 TS

F006 The following crude oils were examined:

- ** Prudhoe Bay crude oil (PBCO) [a heavy crude]
- ** South Louisiana crude oil (SLCO) [a light crude]
- ** Arabian Light crude oil (ALCO)
- ** In addition a bunker C oil was examined
- ** A USP grade mineral oil and a corn

F007 The following crude oils were examined:

- ** Prudhoe Bay crude oil (PBCO) [a heavy crude]
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- ** Arabian Light crude oil (ALCO)
- ** In addition a bunker C oil was examined
- ** A USP grade mineral oil and a corn oil were also included in the studies.

F008 IUC4

F020 4701

EOR

F002 37

F010 5.5

F004 1

F005 CL

F006 A modified Ames assay conducted on five different crudes

- ** demonstrated that all but one (Lost Hills light) were
- ** mutagenic. The Lost Hills Light did not demonstrate any
- ** mutagenic activity.

F007 A modified Ames assay conducted on five different crudes

```
** demonstrated that all but one (Lost Hills light) were
** mutagenic. The Lost Hills Light did not demonstrate any
** mutagenic activity.
F008 IUC4
F020 4702
EOR
F002 37
F010 5.5
F004 1
F005 ME
F006 DMSO extracts were prepared by mixing 2 ml test material
** with 3 ml cyclohexane to homogeneity. 10 ml DMSO was then
   added and mixed thoroughly. The mixture was vortexed every 5
** minutes for a total of 30 minutes. After 30 minutes the
** mixture
F007 DMSO extracts were prepared by mixing 2 ml test material
** with 3 ml cyclohexane to homogeneity. 10 ml DMSO was then
   added and mixed thoroughly. The mixture was vortexed every 5
   minutes for a total of 30 minutes. After 30 minutes the
   mixture was centrifuged and the DMSO layer (extract) removed
   and stored for testing.
** The extract was only tested with metabolic activation in
** Salmonella typhimurium strain TA 98 at the following doses:
^{**} 1, 3, 5, 7, 10, 15, 25 and 50 \mu l/plate. Additionally the
** DMSO extract of a carcinogenic oil was also tested in the
** same manner.
** Positive control chemicals were: 2-aminoanthracene (2 ug)and
   benzoa(a)pyrene (5 ug).
** The DMSO extract of a refrigerator oil was use as negative
** control (50 ul).
** The metabolic activation mixture was derived from
   Araclor-induced hamster liver and this was use at eight
   times the standard concentration (0.4 ml rather than 0.05
   ml).
** The test material and control substances were added to tubes
   at the doses shown above and were incubated for 20 minutes
   with Salmonella broth culture. Colonies of histidine
   prototrophs were counted 48 hours after plate incubation.
   Assay acceptance criteria
                A linear dose response curve for mutagenicity must be
                obtained before the test material can be ranked for
                potency
                                                                                                TA98
                Spontaneous and solvent control reversion rates for
   must fall between 20 and 50 revertants /plate
**
               The slope of the dose response curve for the positive
               control carcinogenic oil must fall between 1.5 - 4.5
                                                                                net
   revertants/plate
```

- ** Provided the above criteria are met the net revertants per
- ** plate is plotted against dose and the best initial straight
- ** line response is determined by regression analysis.
- ** Test materials producing slopes less than 0.5 net
- ** revertants/µI are considered inactive.

F020 4703

EOR

F002 37

F010 5.5

F004 1

F005 RE

F006 Mobil (1984)

- ** A modified Ames pre-incubation assay for the determination
- ** of specific mutagenicity of the DMSO extract of Arab light
- ** crude
- ** Study No. 40965
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1984)

- ** A modified Ames pre-incubation assay for the determination
- ** of specific mutagenicity of the DMSO extract of Arab light
- ** crude
- ** Study No. 40965
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4704

EOR

F002 37

F010 5.5

F004 1

F005 RE

F006 Mobil (1984)

- ** A modified Ames pre-incubation assay for the determination
- ** of specific mutagenicity of the DMSO extract of MCSL crude
- ** (Midcontinent)
- ** Study No. 40975
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1984)

- ** A modified Ames pre-incubation assay for the determination
- ** of specific mutagenicity of the DMSO extract of MCSL crude
- ** (Midcontinent)
- ** Study No. 40975
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4705

EOR

F002 37

F010 5.5

F004 1

F005 RE

F006 Mobil (1984)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract

- ** of Beryl crude
- ** Mobil Health and Environmental Science Laboratory Study
- ** 40955

F007 Mobil (1984)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract
- ** of Beryl crude
- ** Mobil Health and Environmental Science Laboratory Study
- ** 40955

F008 IUC4

F020 4706

EOR

F002 37

F010 5.5

F004 1

F005 RE

F006 Mobil (1990)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract
- ** of Belridge Heavy
- ** Study No. 63850
- ** Mobil Health and Environmental Science Laboratory

F007 Mobil (1990)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract
- ** of Belridge Heavy
- ** Study No. 63850
- ** Mobil Health and Environmental Science Laboratory

F008 IUC4

F020 4707

EOR

F002 37

F010 5.5

F004 1

F005 RE

F006 Mobil (1990)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract
- ** of Lost Hills Light
- ** Study No. 63838
- ** Mobil Health and Environmental Science Laboratory

F007 Mobil (1990)

- ** A modified Ames pre-incubation mutagenesis assay for
- ** determination of specific mutagenicity of the DMSO extract
- ** of Lost Hills Light
- ** Study No. 63838
- ** Mobil Health and Environmental Science Laboratory

F008 IUC4

F020 4708

EOR

F002 37

F010 5.5

F004 1

F005 RL

F006 It is not clear whether the studies were carried out

- ** according to GLP. Furthermore, the assay was designed as a
- ** screen for carcinogenic activity However, the results are
- ** useful to identify the mutagenic potential of crude oils.

F007 It is not clear whether the studies were carried out

- ** according to GLP. Furthermore, the assay was designed as a
- ** screen for carcinogenic activity However, the results are
- ** useful to identify the mutagenic potential of crude oils.

F008 IUC4

F020 4709

EOR

F002 37

F010 5.5

F004 1

F005 RM

F006 Similar studies were conducted for Arab light, MCSL, Lost

- ** Hills light and Belridge Heavy crudes.
- ** The results were:

**

**	Crude	Slope	Reference
**	Arab light 3.8		Mobil study 40965
**	MCSL crude 1.5		Mobil study 40975
**	Relridge Heavy	1 7	Mohil et

F007 Similar studies were conducted for Arab light, MCSL, Lost

- ** Hills light and Belridge Heavy crudes.
- ** The results were:

*

**	Crude		Slope	Reference
**	Arab light	3.8		Mobil study 40965
**	MCSL crude	1.5		Mobil study 40975

* Belridge Heavy 1.7 Mobil study 663850

Lost Hills light 0 Mobil study 63838

F008 IUC4

F020 4710

EOR

F002 37

F010 5.5

F004 1

F005 RS

F006 The No. revertants for each dose level of Beryl crude are as

** follows (NB data for the carcinogenic oil are not shown):

**

**	Dose (µI)	No. of revertar	nts	
**	0 (Spontaneo	us)		43
**	0 (solvent co	ntrol)	39	
**	50			113
**	25			85
**	15			74
**	10			67
**	7			61

F007 The No. revertants for each dose level of Beryl crude are as

^{**} follows (NB data for the carcinogenic oil are not shown):

**			
**	Dose (µI) No. of revertan	ts	
**	0 (Spontaneous)		43
**	0 (solvent control)	39	
**	50		113
**	25		85
**	15		74
**	10		67
**	7		61
**	5		57
**	3		47
**	1		48
**	2-AA control	540	
**	BaP control	366	
**	Refrigerator oil	43	
**			
**	The slope for the Beryl crude was determin	ed to be 2.5	

- ** The slope for the carcinogenic oil was 3.7

F020 4711

EOR

F002 37

F010 5.5

F004 2

F005 ME

F006 Preparation of DMSO extract

- ** 10 ml DMSO was added to approximately 2 ml test substance.
- ** The tube containing the mixture was vortexed every 5 minutes for one
- minute. After 30 minutes, the mixture was
- ** centrifuged and the DMSO layer (Extract) w

F007 Preparation of DMSO extract

- ** 10 ml DMSO was added to approximately 2 ml test substance.
- ** The tube containing the mixture was vortexed every 5 minutes for one
- minute. After 30 minutes, the mixture was
- centrifuged and the DMSO layer (Extract) was removed for
- testing.

Control substances:

Solvent control: **DMSO**

Negative control: Untreated flasks

Positive controls: Cyclophosphamide monohydrate at

concentration of 10 µg/ml

Duplicate cultures were used - one set for metaphase

- analysis and the other for a determination of cytotoxicity
- (Mitotic Index). All cultures were conducted with metabolic
- activation.

- ** CHO cells were exposed for two hours to test material at
- ** concentrations of 1, 2.5, 5, 10, 15 and 20 µl/ml.
- ** After two hours, treatment was terminated and the cultured
- cells were washed and re-fed with complete culture medium.
- ** Approximately 16 hours later, colcicine was added and two

hours later the cells were harvested as described below. Metaphase analysis Metaphase cells were collected by tapping a flask to release the loosely attached mitotic cells into culture medium and pelleting them by centrifugation. The cells were resuspended and then fixed in methanol:acetic acid (3:1) and stored refrigerated until slide preparation. A minimum of 4 slides was made for each culture flask. 100 cells were examined microscopically per flask and were scored for structural chromosomal aberrations. Although gaps were recorded they were not used in the analysis for chromosomal aberration since their significance is questionable. ** Cytotoxicity assay (Mitotic Index determination) For this portion of the assay all cells in each flask were ** collected. The medium in each flask was replaced and 5 ml of trypsin was added and after incubation for approximately 5 minutes the flasks were examined to ensure that the cells had rounded up. The cells were then released into the trypsin solution mechanically and the resulting suspension was centrifuged in the presence of medium. The centrifuged cells were swelled in hypotonic solution and were fixed in methanol:acetic acid as above. A minimum of 2 slides per flask were prepared for the determination of mitotic index. The slides were stained with Giemsa and at least 1000 nuclei per flask were scored as either mitotic or interphasic. The MI = No mitotic cells x 100/Total No of cells scored.** Criteria for a positive or negative response ** A test substance is considered to have elicited a positive response if at least one concentration shows a statistically significant increase in the proportion of cells with aberrations and a significant positive dose-response exists. ** Biological significance of the result is also taken into ** account in determining the response. F008 IUC4 F020 4712 **EOR** F002 37 F010 5.5

F004 2 F005 RE

F006 Mobil (1991)

** Study 63840

F007 Mobil (1991)

** Metaphase analysis of Chinese hamster ovary (CHO) cells
 ** treated in-vitro with a DMSO extract of Lost Hills Light

** Mobil Environmental and Health Science Laboratory

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** Metaphase analysis of Chinese hamster ovary (CHO) cells
** treated in-vitro with a DMSO extract of Lost Hills Light
** Study 63840
** Mobil Environmental and Health Science Laboratory
F008 IUC4
F020 4713
EOR
F002 37
F010 5.5
F004 2
F005 RL
F006 Despite the fact that whether the study was carried out to
** GLP is not clear, it is, nevertheless, reliable and valid.
F007 Despite the fact that whether the study was carried out to
** GLP is not clear, it is, nevertheless, reliable and valid.
F008 IUC4
F020 4714
EOR
F002 37
F010 5.5
F004 2
F005 RS
F006 Determination of Mitotic Index (MI)
** There was not a dose related decrease in MI at treatments
   that were not toxic. The MI at each of the dose
   concentrations of Lost Hills Light crude (LHL)was:
** Treatment
                                                                 Mitotic Index
** 20 µl/ml extract o
F007 Determination of Mitotic Index (MI)
** There was not a dose related decrease in MI at treatments
   that were not toxic. The MI at each of the dose
   concentrations of Lost Hills Light crude (LHL)was:
** Treatment
                                                                 Mitotic Index
** 20 µl/ml extract of LHL
                                                                 no cells found
** 15 µl/ml extract of LHL
                                                                 no cells found
** 10 µl/ml extract of LHL
                                                                 0.6
** 5 µl/ml extract of LHL
                                                                 10.7
** 2.5 µl/ml extract of LHL
                                                8.4
   1 µl/ml extract of LHL
                                                                 12.2
   20 µl/ml DMSO
                                                                 7.6
                                                 10.4
   Negative control
   Metaphase analysis
^{\star\star}   

The concentrations analyzed for chromosomal aberrations were 5, 2.5 and
```

** There was no increase in the proportion of cells with

```
structural chromosome aberrations (gaps excluded). Nor was a dose
   response observed.
   The data are:
   Treatment
                                                                No of cells with
                                                                one or more
                                                                aberrations
                                                                (100 cells examined)
   5 µl/ml extract of LHL
                                                                2
   2.5 µl/ml extract of LHL
                                                2
   1 µl/ml extract of LHL
   DMSO (solvent control
                                                                2
                                                2
   Negative control
** CP (positive control)
                                                28
F008 IUC4
F020 4715
EOR
F002 37
F010 5.5
F004 3
F005 ME
F006
** Preparation of DMSO extract
** 10 ml DMSO was added to approximately 2 ml test substance.
** The tube containing the mixture was vortexed every 5 minutes for one
   minute. After 30 minutes, the mixture was
** centrifuged and the DMSO layer (Extract)
F007
** Preparation of DMSO extract
** 10 ml DMSO was added to approximately 2 ml test substance.
** The tube containing the mixture was vortexed every 5 minutes for one
   minute. After 30 minutes, the mixture was
   centrifuged and the DMSO layer (Extract) was removed for
   testing.
** Control substances:
   Solvent control:
                                DMSO
   Negative control:
                                Untreated flasks
   Positive controls:
                                Cyclophosphamide monohydrate at
                                               concentration of 10 µg/ml
   Duplicate cultures were used - one set for metaphase
   analysis and the other for a determination of cytotoxicity
   (Mitotic Index). All cultures were conducted with metabolic
   activation.
** CHO cells were exposed for two hours to test material at
   concentrations of 1, 2.5, 5, 10, 15 and 20 \mul/ml.
   After two hours, treatment was terminated and the cultured
```

** cells were washed and re-fed with complete culture medium.

- ** Approximately 16 hours later, colcicine was added and two
- ** hours later the cells were harvested as described below.

**

- ** Metaphase analysis
- ** Metaphase cells were collected by tapping a flask to release the loosely
- * attached mitotic cells into culture medium and pelleting them by
- * centrifugation. The cells were resuspended and then fixed in
- * methanol:acetic acid (3:1) and stored refrigerated until slide
- preparation.
- ** A minimum of 4 slides was made for each culture flask.
- ** 100 cells were examined microscopically per flask and were
- ** scored for structural chromosomal aberrations. Although gaps were
- * recorded they were not used in the analysis for
- ** chromosomal aberration since their significance is
- ** questionable.

**

- ** Cytotoxicity assay (Mitotic Index determination)
- ** For this portion of the assay all cells in each flask were
- ** collected.
- ** The medium in each flask was replaced and 5 ml of trypsin
- ** was added and after incubation for approximately 5 minutes
- ** the flasks were examined to ensure that the cells had
- ** rounded up.
- ** The cells were then released into the trypsin solution
- ** mechanically and the resulting suspension was centrifuged in the presence
- * of medium. The centrifuged cells were swelled in hypotonic solution and
- * were fixed in methanol:acetic acid as above.
- ** A minimum of 2 slides per flask were prepared for the
- ** determination of mitotic index.
- ** The slides were stained with Giemsa and at least 1000 nuclei per flask
- * were scored as either mitotic or interphasic.

**

** The MI = No mitotic cells x 100/Total No of cells scored.

*

- ** Criteria for a positive or negative response
- ** A test substance is considered to have elicited a positive
- ** response if at least one concentration shows a statistically
- ** significant increase in the proportion of cells with
- ** aberrations and a significant positive dose-response exists.
- ** Biological significance of the result is also taken into
- ** account in determining the result.

F008 IUC4

F020 4716

EOR

F002 37

F010 5.5

F004 3

F005 RE

F006 Mobil (1991)

- ** Metaphase analysis of Chinese hamster ovary (CHO) cells
- ** treated in-vitro with a DMSO extract of Belridge Heavy
- ** Study 63852
- ** Mobil Environmental and Health Science Laboratory

```
F007 Mobil (1991)
** Metaphase analysis of Chinese hamster ovary (CHO) cells
** treated in-vitro with a DMSO extract of Belridge Heavy
** Study 63852
** Mobil Environmental and Health Science Laboratory
F008 IUC4
F020 4717
EOR
F002 37
F010 5.5
F0043
F005 RL
F006 Despite the fact that whether the study was carried out to
** GLP is not clear, it is, nevertheless, reliable and valid.
F007 Despite the fact that whether the study was carried out to
** GLP is not clear, it is, nevertheless, reliable and valid.
F008 IUC4
F020 4718
EOR
F002 37
F010 5.5
F0043
F005 RS
F006 Determination of Mitotic Index (MI)
** There was not a dose related decrease in MI at treatments
   that were not toxic. The MI at each of the dose
   concentrations of Belridge Heavy crude (BH)was:
** Treatment
                                                                Mitotic Index
** 20 µl/ml extract of B
F007 Determination of Mitotic Index (MI)
** There was not a dose related decrease in MI at treatments
** that were not toxic. The MI at each of the dose
   concentrations of Belridge Heavy crude (BH)was:
** Treatment
                                                                Mitotic Index
** 20 µl/ml extract of BH
                                                                no cells found
** 15 µl/ml extract of BH
                                                                no cells found
** 10 µl/ml extract of BH
                                                                no cells found
** 5 µl/ml extract of BH
                                                11.2
   2.5 µl/ml extract of BH
                                                                10.2
   1 µI/ml extract of BH
                                                13.1
                                                                7.6
   20 µl/ml DMSO
   Negative control
                                                10.4
   Metaphase analysis
```

** The concentrations analyzed for chromosomal aberrations were

** 5, 2.5 and 1.0 μg/ml.

```
structural chromosome aberrations (gaps excluded). Nor was a dose
   response observed.
   The data are:
   Treatment
                                                               No of cells with
                                                               one or more
                                                               aberrations
                                                               (100 cells examined)
** 5 µl/ml extract of LHL
   2.5 µl/ml extract of LHL
                                               2
   1 µl/ml extract of LHL
                                                               1
**
   DMSO (solvent control
                                                               2
   Negative control
                                               2
** CP (positive control)
                                               28
F008 IUC4
F020 4719
EOR
F002 37
F010 5.5
F004 4
F005 ME
F006 The authors summarized the protocol in the following table:
** Test strains
   Salmonella typhimurium strains TA-1535, TA-1537, TA-1538,
   TA-98 and TA-100
   Liver homogenate
** S-9 from male Charles River CD rat liver
   500 mg Aroclor/kg for 5 days
F007 The authors summarized the protocol in the following table:
** Test strains
   Salmonella typhimurium strains TA-1535, TA-1537, TA-1538,
   TA-98 and TA-100
** Liver homogenate
** S-9 from male Charles River CD rat liver
** 500 mg Aroclor/kg for 5 days
** Procedure
** Treatment without activation: 2 ml top agar, 0.1 ml
   dissolved test chemical, 0.1 ml bacterial culture (108
   cells)
```

** Treatment with activation: 2 ml top agar, 0.1 ml dissolved

** There was no increase in the proportion of cells with

```
ml S-9 mix contains per milliliter 0.3 ml S-9 (1 g tissue +
   3 ml 0.15M KCl), 8 mM Mg Cl2, 33 mM KCl, 5mM
   glucose-6-phosphate, 4 mM NADP, and 100 mM sodium phosphate
   (pH 7.4).
   Incubation period: 48 hr
   Design: Preliminary toxicity determination
        Duplicate plates/point
        Five test concentrations and solvent and positive
                controls
        Duplicate experiments
   Solvent
   Dimethyl sulfoxide
   Data analysis
   Mutagenic: statistically significant. Increase in total
   revertant colony number (P</= 0.01) and dose response
   (P < /= 0.01)
   Scoring
                                                Induced
                                                frequency/
                                                Spontaneous
**
                Revertant/µg
                                                frequency
** (-)
                < 0.01
                0.1 to 0.01
   (+)
                                <5
                >0.1
                                                >5
   (++)
** * No greater than response
F008 IUC4
F020 4720
EOR
F002 37
F010 5.5
F004 4
F005 RE
F006 Calkins, W. H., Deye, J. F., Hartgrove, R. W., King, C. F.
** and Krahn, D. F. (1981)
** Synthetic crude oils from coal: Mutagenicity and
** tumor-initiating screening tests.
** In Mahlum, D. D. et al (Eds)
** Coal conversion and the environment pp 461-47
F007 Calkins, W. H., Deye, J. F., Hartgrove, R. W., King, C. F.
** and Krahn, D. F. (1981)
** Synthetic crude oils from coal: Mutagenicity and
** tumor-initiating screening tests.
** In Mahlum, D. D. et al (Eds)
** Coal conversion and the environment pp 461-470
** U. S. Dept. of Energy, Washington D. C.
```

** test chemical, 0.1 ml bacterial culture (108 cells) plus 0.5

```
F020 4721
EOR
F002 37
F010 5.5
F004 4
F005 RL
F006 The study is not fully reported (data for the assay without
** metabolic activation are not given)
F007 The study is not fully reported (data for the assay without
** metabolic activation are not given)
F008 IUC4
F020 4722
EOR
F002 37
F010 5.5
F004 4
F005 RS
F006 The results are given in the following table
   Sample
                                               Mutagenic activity
                                               (with activation)
                                               TA-1537
                                                               TA-1538
                                                                               TA-98
                                                                                               TA-100
   Benzo[a]pyrene
                                               ++
                                                               ++
   South Louisiana crude
   It is not clear from the report whether any activity
F007 The results are given in the following table
   Sample
                                               Mutagenic activity
                                               (with activation)
                                                                                               TA-100
                                               TA-1537
                                                               TA-1538
                                                                               TA-98
   Benzo[a]pyrene
                                               ++
                                                               ++
   South Louisiana crude
**
** It is not clear from the report whether any activity was
** detected in the assay without metabolic activation.
F008 IUC4
F020 4723
EOR
F002 37
F010 5.5
F004 5
F005 ME
F006 Salmonella typhimurium strains TA 98 and TA 100 were used in this study.
** The test sample was suspended in Tween 80 prior to testing.
** Each sample was weighed in a glass vial and then an equal
** weight of Tween 80 was added. The oil and deterge
```

F007 Salmonella typhimurium strains TA 98 and TA 100 were used in this study.

** The test sample was suspended in Tween 80 prior to testing.
** Each sample was weighed in a glass vial and then an equal
** weight of Tween 80 was added. The oil and detergent were

- ** stirred until thoroughly homogenized. Distilled water was
- ** then added dropwise with continuous stirring until a stable, homogenous
- * suspension was obtained. The concentration of the mixture was then
- * adjusted to 10% sample 10% Tween 80 (w/v) by the addition of more
- distilled water.
- ** The assay was carried out in triplicate with and without
- ** metabolic activation by S9 liver homogenate that was
- ** prepared from Aroclor-induced Sprague-Dawley rats. The S9
- ** homogenate contained 13 mg of protein/ml and was stored at
- ** -70°C. S9 homogenate was used at a concentration of 10%
- ** (v/v) in the assay.
- ** BaP was used as the positive control.
- ** Negative control plates for determination of the spontaneous reversion
- * rate were treated with 400µl or less of 10% Tween 80, a non-toxic and
- * non-mutagenic dose.

**

- ** No other experimental details are given in the paper, but
- ** reference is made to the papers by Ames that describe the
- ** methodology.

F008 IUC4

F020 4724

EOR

F002 37

F010 5.5

F004 5

F005 RE

F006 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived
- ** oil
- ** Mutation Research Vol 102, pp

F007 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived
- ** oil
- ** Mutation Research Vol 102, pp 221-235

F008 IUC4

F009 28-08-2003

F020 4725

EOR

F002 37

F010 5.5

F004 5

F005 RL

F006 The numbers of revertants were not given. The result was

** simply stated by the authors.

F007 The numbers of revertants were not given. The result was

** simply stated by the authors.

F008 IUC4

F020 4726

EOR

```
F002 37
F010 5.5
F004 5
F005 RS
F006 Addition of up to 400µl Tween 80 per plate did not alter the
** spontaneous reversion rates of either TA 98 or TA 100. Tween
** 80 had no effect on the mutagenicity of BaP, which induced
   similar numbers of revertants in TA 98 with S9 activation
F007 Addition of up to 400µl Tween 80 per plate did not alter the
** spontaneous reversion rates of either TA 98 or TA 100. Tween
** 80 had no effect on the mutagenicity of BaP, which induced
** similar numbers of revertants in TA 98 with S9 activation
** whether dissolved in DMSO or Tween 80.
** The crude oil sample was inactive in all bacterial
** mutagenicity tests.
F008 IUC4
F020 4727
FOR
```

F005 ME
F006 Heparinized blood was obtained from a healthy 25-year old

** male. [The method for culture of the lymphocytes was not

** described but reference was given which describes the

** method].

F002 37 F010 5.5 F004 6

** Benzo(a)pyrene (BaP) and N-methyl-N'-nitro-N-nitroso

** guanidine

F007 Heparinized blood was obtained from a healthy 25-year old

** male. [The method for culture of the lymphocytes was not

** described but reference was given which describes the

** method].

** Benzo(a)pyrene (BaP) and N-methyl-N'-nitro-N-nitroso

** guanidine (MNNG) were used as positive control substances.

** The test sample was suspended in Tween 80 prior to testing.

* Each sample was weighed in a glass vial and then an equal

** weight of Tween 80 was added. The oil and detergent were

** stirred until thoroughly homogenized. Distilled water was

** then added dropwise with continuous stirring until a stable, homogenous

* suspension was obtained. The concentration of the mixture was then

* adjusted to 1% sample - 1% Tween 80 (w/v) by the addition of more

distilled water.

** Lymphocyte cultures were treated 18 hours after initiation

** by (a) continuous exposure to test chemical without

** exogenous activation or

** (b) by exposure for 2.5 hours with activation by

** [procedure described elsewhere by Stetka and Wolff (1976)

** for metabolic activation].

** In tests with S9, cells were collected by centrifugation at

** 150 x g for 10 minutes, resuspended in 5 ml of McCoy's 5A

** medium containing only antibiotics and treated with

S9.

- ** microliter quantities of test material or solvent. An
- ** aliquot of 0.25 ml of S9 mix containing 10% S9 (v/v)
- ** was added to each culture. Cells were incubated for 2.5
- ** hours with occasional mixing. The cells were collected,
- ** washed once in medium with 5% fetal bovine serum and
- ** resuspended in 8 ml of culture medium containing BrdUrd for
- ** further incubation. In experiments testing MNNG, lymphocytes
- ** that had been cultured for 18 hours were suspended in
- ** medium without serum, S9 or BrdUrd, treated for 2 hours with MNNG in
- * acetone or acetone alone (controls), washed as
- ** above, resuspended in complete medium with BrdUrd and
- ** incubated further. At 68 hours after the cultures were
- ** initiated, Colcemid (0.01µg/ml) was added; 4 hours later,
- ** the cells were harvested by centrifugation, subjected to
- ** hypotonic shock for 7 minutes in 0.075M KCl, fixed in 3
- ** changes of cold methanol-acetic acid (3:1) and spread on
- ** chilled, wet microslides that had been pre-cleaned in 95%
- ** ethanol. The preparations were stained by the Hoechst
- ** 33258-black light-Giemsa method and numbers of SCE were
- ** counted in 25 metaphase cells containing 40-48 chromosomes
- ** for each treatment.

F020 4728

EOR

F002 37

F010 5.5

F004 6

F005 RE

F006 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived
- ** oil
- ** Mutation Research Vol 102, pp

F007 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived
- ** oil.
- ** Mutation Research Vol 102, pp 221-235

F008 IUC4

F009 28-08-2003

F020 4729

EOR

F002 37

F010 5.5

F004 6

F005 RL

F006 Complete description of study not given in publication. Some

** methods details published elsewhere

F007 Complete description of study not given in publication. Some

** methods details published elsewhere

F008 IUC4

F020 4730

EOR

F002 37

F010 5.5

F004 6

F005 RS

F006 Tween 80 was not cytotoxic and did not induce increases in

- ** the numbers of SCE in cultures of lymphocytes treated with
- ** 100 ppm Tween 80 for 54 hours without activation or for 2.5
- ** hours with S9 activation.
- ** MNNG, a strong alkylating agent, ind

F007 Tween 80 was not cytotoxic and did not induce increases in

- ** the numbers of SCE in cultures of lymphocytes treated with
- ** 100 ppm Tween 80 for 54 hours without activation or for 2.5
- ** hours with S9 activation.
- ** MNNG, a strong alkylating agent, induced a greater than
- ** 5-fold increase in SCE at a concentration of only 0.15 ppm.
- ** The response to BaP in the lymphocyte cultures indicated
- ** that the S9 metabolic system was functional. Although
- ** increases in SCE of only 50% over the number in control
- ** cultures were induced, these increases are statistically
- ** significant (P<0.001). In the presence of S9 mix, only 1/16
- ** the amount of BaP was required to induce the same number of
- ** SCE observed without S9, indicating that metabolism had
- ** occurred. However, 0.5µg/ml of BaP with S9 mix was toxic to
- ** these cultures, as indicated by poor cell morphology and a
- ** reduced number of cells in mitosis.
- ** Wilmington crude did not induce an increase in SCE at any
- ** dose concentration with or without S9 activation.
- ** The data are summarized in the following table.

**				
**	Treatment	Dose	No. of	SCE/cell
**		(ppm)	cells	±S.E.
**				
**	24 hours exposure, without \$	59		
**	Tween 80	0	25	8.4±0.5
**		100	25	9.7±0.6
**				
**	B(a)P	0	25	7.7±0.6
**		4	25	10.8±1.2
**		8	25	15.1±0.9**
**				
**	Wilmington			
**	crude oil	40	25	10.7±1.2
**		50	25	9.9±0.8
**				
**	2.5 hours exposure, with S9			
**	Tween 80	40	25	9.6±0.0
**		100	25	8.0±0.5
**				
**	B(a)P	0	25	10.9±1.9
**		0.2	25	13.4±0.8**
**		0.5	10	15.9±1.2**

**		1.0	0	Toxic
**				
**	MNNG	0	25	8.7±0.4
**		0.015	23	11.7±0.5**
**		0.05	23	28.3±1.3**
**		0.15	21	48.5±2.2**
**				
**	Wilmington			
**	crude oil	20	25	8.8±0.7
**		30	25	6.6±0.5
**				
**	**	P<0.001		

F020 4731

EOR

F002 37

F010 5.6

F004 1

F005 ME

F006 The animals used in this study were taken from a 13 week

- ** repeated dermal administration study (Study 63834).
- ** Bone marrow was removed from the femurs of five male and
- ** five female rats from each dose group (0, 30, 125 and 500
- ** mg/kg/day).
- ** A ne

F007 The animals used in this study were taken from a 13 week
** repeated dermal administration study (Study 63834).

- ** Bone marrow was removed from the femurs of five male and
- ** five female rats from each dose group (0, 30, 125 and 500
- ** mg/kg/day).
- ** A nearly pure erythrocyte fraction was obtained and two
- ** slides prepared for each animal.
- ** 1000 PCEs (polychromatic erythrocyte) and 1000 NCEs
- ** (normochromatic erythrocyte) were scored to determine the
- ** percentage of micronucleated erythrocytes. 1000 erythrocytes
- ** were scored to determine the ratio of PCEs to NCEs.

F008 IUC4

F020 4732

EOR

F002 37

F010 5.6

F004 1 F005 RE

F006 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived
- ** oil.
- ** Mutation Research Vol 102, pp

F007 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and

- ** Sabharwal. P. S. (1982)
- ** Comparative study of the genotoxic properties of Eastern and
- ** Western U. S. shale oils, crude petroleum and coal-derived

- ** oil.
- ** Mutation Research Vol 102, pp 221-235

F009 28-08-2003

F020 4733

EOR

F002 37

F010 5.6

F004 1

F005 RE

F006 Mobil (1990)

- ** Micronucleus assay of bone marrow red blood cells from rats
- ** treated via dermal administration of Lost Hills Light
- ** Study 63835
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1990)

- ** Micronucleus assay of bone marrow red blood cells from rats
- ** treated via dermal administration of Lost Hills Light
- ** Study 63835
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4734

EOR

F002 37

F010 5.6

F004 1

F005 RE

F006 Mobil (1991)

- ** Micronucleus assay of bone marrow cells from rats
- ** treated via dermal administration of Belridge Heavy
- ** Study 63847
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1991)

- ** Micronucleus assay of bone marrow cells from rats
- ** treated via dermal administration of Belridge Heavy
- ** Study 63847
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4735

EOR

F002 37

F010 5.6

F004 1

F005 RE

F006 Mobil (1992)

- ** Thirteen-week dermal administration of Lost Hills Light to
- ** rats
- ** Study No. 63834
- ** Environmental Health and Sciences Laboratory

F007 Mobil (1992)

- ** Thirteen-week dermal administration of Lost Hills Light to
- ** rats
- ** Study No. 63834

```
** Environmental Health and Sciences Laboratory
F008 IUC4
F020 4736
EOR
F002 37
F010 5.6
F004 1
F005 RM
F006 Two further studies have been reported.
                Belridge heavy crude.
                The results of this study were the same as those
                                                                                                for the study on Lost
   Hills Light described above.
                                                                (Mobil study 63847)
   2.
                Wilmington crude
                Results in ICR Swiss mice s
F007 Two further studies have been reported.
  1.
                Belridge heavy crude.
                The results of this study were the same as those
                                                                                                for the study on Lost
   Hills Light described above.
                                                                (Mobil study 63847)
   2.
                Wilmington crude
                Results in ICR Swiss mice showed that Wilmington
                                                                                                crude oil had
   relatively little capacity to cause
                                                                chromosome breakage and/or
                               (Lockard et al, 1982)
   non-disjunction.
F008 IUC4
F020 4737
EOR
F002 37
F010 5.6
F004 1
F005 RS
F006 The results are given in the following table. Standard
   deviations have not been included in the table. However,
   statistical analyses were conducted and it was found that
   there were no significant differences between treated and
   control anim
F007 The results are given in the following table. Standard
   deviations have not been included in the table. However,
   statistical analyses were conducted and it was found that
** there were no significant differences between treated and
   control animals.
   It was concluded that the test material was not cytotoxic to
   red blood cell formation and furthermore did not increase
   the formation of micronucleated PCEs or NCEs in the bone
   marrow.
                Sex
                                No of
                                                PCE/NCE
                                                                MNPCEs
                                                                                MNNCEs
   Dose
                                animals
   mg/kg
                                                                (%)
                                                                                (%)
```

** 0

5

1.34

0.1

0

**	0	M	5	0.79	0.02	0		
**	0	M+F	10	1.06	0.06	0		
**								
**	30	F	5	1.26	0.04	0		
**	30	M	5	0.68	0	0.02		
**	30	M+F	10	0.97	0.02	0.01		
**								
**	125	F	5	1.18	0	0		
**	125	M	5	1.17	0.06	0		
**	125	M+F	10	1.17	0.03	0		
**								
**	500	F	5	1.25	0.02	0		
**	500	M	5	0.83	0.04	0		
**	500	M+F	10	1.04	0.03	0		
**								
**		PCE	Polychromatic erythrocytes					
**		NCE	Normochromatic erythrocytes					
**		%MNPCEs	% Micronucleated PCEs					
**		%MNNCEs	% Micronucleated normochromatic erythrocytes					

F020 4738

EOR

F002 37

F010 5.6

F004 2

F005 ME

F006 The assay was carried out in groups of 3 male 10-14 week old Sch:ICR mice.

- ** The mice were lightly anesthetized with ether and a 50 mg
- ** tablet of BrdUrd was inserted beneath the skin on the dorsal side of the
- * neck of each animal. After 1 hour,

F007 The assay was carried out in groups of 3 male 10-14 week old Sch:ICR mice.

- ** The mice were lightly anesthetized with ether and a 50 mg
- ** tablet of BrdUrd was inserted beneath the skin on the dorsal side of the
- * neck of each animal. After 1 hour, treatment was given by a single i.p.
- * injection at doses of 1.8, 3.6 and 7.2 g/kg (equivalent to 0.8, 0.4 and
- * 0.2 times the approximate LD50).
- ** Control groups of mice were injected with B(a)P at doses of
- ** $\,$ 0.16, 0.08 and 0.04 g/kg, Cyclophosphamide at doses of $\,$ 0.01 and 0.005 $\,$
- * g/kg in distilled water. Trioctanoin and distilled water were also given
- * to negative solvent controls.
- ** 22 hours after treatment, animals were injected i.p. with
- ** Colchicin (10 mg/kg). 2 hours later the mice were sacrificed by cervical
- * dislocation and their femurs were removed. The bone marrow cells were
- * flushed out with saline. Pooled cells from the 2 femurs of each animal
- * were treated with hypotonic KCI for 30 minutes, were fixed and were
- spread on slides.
- ** The slides were stained and the numbers of SCE counted in 25 metaphase
- * cells from each animal, each metaphase having
- ** 36-42 chromosomes.

F008 IUC4

F020 4739

EOR

F002 37

```
F010 5.6
F004 2
F005 RE
F006 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and
** Sabharwal. P. S. (1982)
** Comparative study of the genotoxic properties of Eastern and
** Western U. S. shale oils, crude petroleum and coal-derived
** Mutation Research Vol 102, pp
F007 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and
** Sabharwal. P. S. (1982)
** Comparative study of the genotoxic properties of Eastern and
** Western U. S. shale oils, crude petroleum and coal-derived
** Mutation Research Vol 102, pp 221-235
F008 IUC4
F009 28-08-2003
F020 4740
EOR
F002 37
F010 5.6
F004 2
F005 RL
F006 Probably not to GLP and method description is not
** comprehensive.
F007 Probably not to GLP and method description is not
** comprehensive.
F008 IUC4
F020 4741
EOR
F002 37
F010 5.6
F004 2
F005 RS
F006 Control animals injected with the solvent trioctanoin had a
** mean of 5.3 SCE/cell. A slight but significant increase in
** SCE was found in animals treated with the highest dose only
** of Wilmington crude oil, but the response was not dose
** relate
F007 Control animals injected with the solvent trioctanoin had a
** mean of 5.3 SCE/cell. A slight but significant increase in
** SCE was found in animals treated with the highest dose only
** of Wilmington crude oil, but the response was not dose
** related.
   B(a)P and cyclophosphamide induced dose-related increases in SCE.
   The actual results are shown in the following table.
   Treatment
                Dose No. survivors/ No. cells SCE/
                No. treated examined cell±S.E.
   Trioctanoin 0.5 ml/
           mouse
                      7/8
                                 175
                                      5.3±0.4
```

```
9.7±0.6***
B(a)P
         0.16 g/kg 3/3
                              75
       0.08
                3/3
                          75
                                8.5±0.5***
                                7.8±0.5***
       0.04
                3/3
                          75
Cyclophosphamide
       0.01 g/kg
                 3/3
                            75
                                19.5±0.6***
       0.005
                 3/3
                           75
                               14.6±0.6***
Wilmington crude oil
                           75 6.6±0.4**
      7.2 g/kg
       3.6
                3/3
                          50 5.0±0.3
       1.8
                3/3
                          75 6.4±0.4
                          P<0.05
           ***
                          P<0.001
```

F020 4742

EOR

F002 37

F010 5.7

F004 1

F005 ME

F006 50 mg undiluted test material was applied to the shorn

- ** interscapular region of groups of 50 male mice.
- ** Application was twice weekly for 18 months or until
- ** grossly-observable cancer was found.
- ** Each animal was observed weekly for the appearan

F007 50 mg undiluted test material was applied to the shorn

- ** interscapular region of groups of 50 male mice.
- ** Application was twice weekly for 18 months or until
- ** grossly-observable cancer was found.
- ** Each animal was observed weekly for the appearance of
- ** tumors. The percentage of animals developing tumors and the
- ** time to appearance of first tumor were recorded.
- ** No distinction was made between histologically benign or
- ** malignant lesions.

F008 IUC4

F020 4743

EOR

F002 37

F010 5.7

F004 1

F005 RE

F006 Lewis, S. C., King, R. W., Cragg, S. T. and Hilman, D. W.

- ** (1984
- ** Skin carcinogenesis potential of petroleum hydrocarbons:
- ** II Crude oil, distillate fractions and chemical class
- ** subfractions
- ** In Applied Toxicology of Petroleum Hydrocarbons
- ** Vo

F007 Lewis, S. C., King, R. W., Cragg, S. T. and Hilman, D. W.

- ** (1984)
- ** Skin carcinogenesis potential of petroleum hydrocarbons:

```
** II Crude oil, distillate fractions and chemical class
** subfractions
** In Applied Toxicology of Petroleum Hydrocarbons
** Vol VI of Advances in Modern Environmental Toxicology, M.
** Mehlman, Ed. Princeton Scientific Publishers, Princeton H.J.
** pp 139-150
F008 IUC4
F020 4744
EOR
F002 37
F010 5.7
F004 1
F005 RS
F006 The % tumors and average latencies for the two crudes C & D
** are shown below.
                                              Latency**
                               % Tumors*
   Crude C
                               33
                                                              76
   Crude D
                               56
                                                              64
                               Based on Final Effective Number
                               Average latency in weeks
F007 The % tumors and average latencies for the two crudes C & D
   are shown below.
                               % Tumors*
                                              Latency**
   Crude C
                               33
                                                              76
   Crude D
                               56
                                                              64
                               Based on Final Effective Number
                               Average latency in weeks
F008 IUC4
F020 4745
EOR
F002 37
F010 5.7
F004 1
F005 TS
F006 Two crudes were included in this study. They were
   characterized as follows:
                                                              Crude C
                                                                                             Crude D
** Sulfur (wt%)
                                                              0.21
                                                                                             2.54
** Distillation yield (vol%)
** Int-120°F
                                                              0.0
                                                                                             3.3
** 120-350
                                                              2.5
                                                                                             18.3
** 350-550
                                                                                             19.5
                                                              41.9
** 550-700
                                                              21.0
                                                                                             12.2
F007 Two crudes were included in this study. They were
   characterized as follows:
```

Crude C

Crude D

**	Sulfur (wt%)		0.21		2.54
**	Distillation yield (vol%)				
**	Int-120°F		0.0		3.3
**	120-350		2.5		18.3
**	350-550		41.9		19.5
**	550-700		21.0		12.2
**	700-1070		31.2		26.8
**	1070 btms		3.4		19.9
**					
**	Composition of 550-700°F				
**	Fraction (wt%)		5.1		33.3
**	Monocycloparaffins	8.1		11.5	
**	Polycycloparaffins	46.6		19.5	
**	Total saturated hydrocarbons	59.8		64.3	
**	Mononuclear aromatics		21.5		11.0
**	Di- and Trinuclear aromatics	14.4		21.8	
**	Polynuclear aromatics		1.0		1.8
**	Total aromatic hydrocarbons	36.9		34.6	
**	Resins		3.3		1.1
**	Asphaltenes		0.0		0.0

F020 4746

EOR

F002 37

F010 5.7

F004 2

F005 ME

F006 The study reported by Clarke et al was a comparison of the

- ** carcinogenicity of shale and petroleum crude oil and of
- ** several distillate streams derived from the two crude oils.
- ** Only the details relating to petroleum crude and the
- ** respective p

F007 The study reported by Clarke et al was a comparison of the

- ** carcinogenicity of shale and petroleum crude oil and of
- ** several distillate streams derived from the two crude oils.
- ** Only the details relating to petroleum crude and the
- ** respective positive and negative controls are included in
- ** this summary.

**

- ** The crude oil, positive and negative control materials were
- ** applied three times weekly at a dose of 25 mg/application to 25 male and
- * 25 female mice.
- ** The materials were applied to the shorn dorsal thoracic
- ** region which was shaved as necessary during the study.
- ** Dosing was continued for up to 105 weeks.
- ** Animals were observed twice daily for overt signs of
- ** toxicity, moribundity and mortality. Animals were weighed
- ** weekly for the first 13 weeks and every two weeks
- ** thereafter. Animals were palpated weekly for external and
- ** internal masses.

**

- ** Every animal was subjected to a complete necropsy. All
- ** organs and the remainder of the carcass were fixed and

- ** stored. Body weights and weights of liver, kidneys, brain
- ** and gonads were recorded. A microscopic examination was made of
- * histological slides prepared from skin from a control site, skin from
- * treated site, kidney, liver, lungs, gonads, urinary bladder, spleen,
- * sternal bone marrow and any other organ considered abnormal at necropsy.

F020 4747

EOR

F002 37

F010.5.7

F004 2

F005 RE

F006 Clark, C. R., Walter, M. K., Ferguson, P. W. and Katchen, M.

- ** (1988)
- ** Comparative dermal carcinogenesis of shale and
- ** petroleum-derived distillates
- ** Toxicol. and Ind. Health Vol 4, No. 1, pp 11-22

F007 Clark, C. R., Walter, M. K., Ferguson, P. W. and Katchen, M.

- ** (1988)
- ** Comparative dermal carcinogenesis of shale and
- ** petroleum-derived distillates
- ** Toxicol. and Ind. Health Vol 4, No. 1, pp 11-22

F008 IUC4

F020 4748

EOR

F002 37

F010 5.7

F004 2

F005 RL

F006 Few experimental details are given in this publication.

- ** Despite this, the information may be used in the overall
- ** assessment of the carcinogenic potential of crude oil

F007 Few experimental details are given in this publication.

- ** Despite this, the information may be used in the overall
- ** assessment of the carcinogenic potential of crude oil

F008 IUC4

F020 4749

EOR

F002 37

F010 5.7

F004 2

F005 RS

F006 Survival was affected by treatment with petroleum crude oil.

- ** After one year, survival was approximately 70% and by week
- ** 70 survival was approximately 50%. There were few survivors
- ** at 105 weeks. This is in contrast to the negative control
- ** gr

F007 Survival was affected by treatment with petroleum crude oil.

- ** After one year, survival was approximately 70% and by week
- ** 70 survival was approximately 50%. There were few survivors
- ** at 105 weeks. This is in contrast to the negative control
- ** group with 50% survival at the end of the study.
- ** Dermal irritation at the test site first appeared at 271

- ** days and males developed irritation earlier than the females [no details
- * given]
- ** Necrosis occurred and was characterized as loss of integrity of the skin
- * with visible cracking, separation and sloughing of skin often revealing
- * underlying mesenchymal tissue.

**

- ** There was no indication of toxic or oncogenic effects on
- ** internal organs.

**

- ** The authors reported the occurrence of reactive hyperplasia
- ** in the spleen and bone marrrow and this was characterized by increased
- * populations of lymphocytes, plasma cells and
- ** neutrophils or granulocyte precursors and was attributed to
- ** the inflammation, necrosis and other tissue alterations
- ** occurring at the test site. [There is no indication in the
- ** publication whether this finding occurred in the group
- ** treated with petroleum crude oil.]

*

- ** The tumor incidences that were recorded are shown in the
- ** following table:

**		Crude oil	B(a)P	Control		
**	Incidence (%)	37/44 (84)	49/49	0/46		
**	Latency (weeks)	62±13		28±4		
**	Squamous cell carcinoma	29 (66)		49 (100)	0	
**	Fibrosarcoma	7 (16)		0		0

F008 IUC4

F020 4750

EOR

F002 37

F010 5.7

F004 2

F005 TS

F006 The San Joaquin Valley crude oil is a heavy crude with the

** following characteristics:

** Gravity (°API) 10.7

** Boiling range (°F) 410-1071 ** Nitrogen (ppm wt) 8150

** Sulfur (ppm wt) 10,510

**

** Due to its high viscosity, the crude oil was administered

F007 The San Joaquin Valley crude oil is a heavy crude with the

** following characteristics:

** Gravity (°API) 10.7

** Boiling range (°F) 410-1071 ** Nitrogen (ppm wt) 8150

** Sulfur (ppm wt) 10,510

**

- ** Due to its high viscosity, the crude oil was administered as
- ** a 2:1 dilution in mineral oil.
- ** Mineral oil USP was used as a negative control
- ** B(a)P (0.15% w/v in mineral oil) was used as a positive
- ** control

F008 IUC4

F020 4751 EOR F002 37 F010 5.8.2

F004 1 F005 ME

F006 Groups of twelve presumed-pregnant Sprague-Dawley rats were

- ** distributed into six groups as shown below. The test
- ** material was applied to the shorn dorsal skin once daily at
- ** the doses shown from day 0 to day 19 of gestation. The
- ** animals were

F007 Groups of twelve presumed-pregnant Sprague-Dawley rats were

- ** distributed into six groups as shown below. The test
- ** material was applied to the shorn dorsal skin once daily at
- ** the doses shown from day 0 to day 19 of gestation. The
- ** animals were shaved weekly and were fitted with collars to
- ** prevent ingestion of the test material.

**

** Group Dose level

** Prenatal groups

** 1 Sham control

** 2 30 mg/kg/day

** 3 125 mg/kg/day

** 4 500 mg/kg/day

** Postnatal groups

** 5 Sham control

** 6 500 mg/kg/day

**

- ** Animals were observed at least once daily throughout
- ** gestation until sacrifice for signs of pathosis, abortion,
- ** premature delivery, dystocia and/or death.
- ** dams and their litters were observed postpartum days 0
- ** through 4. On day 0 postpartum, pups were examined for
- ** external malformations and variations and were observed
- ** daily for the presence of milk in their stomachs.

**

- ** Body weights and food consumption of all prenatal group
- ** females were recorded at regular intervals throughout
- ** gestation and for the post natal groups throughout gestation and body
- * weights only on postpartum days 0 and 4.

k

- ** On day 20 of gestation all prenatal group animals were
- ** sacrificed. The abdominal cavity was exposed and the
- ** reproductive organs examined. Following removal of the
- ** uterus and ovaries the remains were subjected to macroscopic examination
- * and the liver and thymus were weighed.

**

- ** The uterus and ovaries of each animal were examined grossly.
- ** The no. of corpora lutea per ovary was recorded. Ovaries of
- ** non pregnant females were examined and then discarded.
- ** The uterine contents of each pregnant animal were exposed
- ** and the number and location of all implantations were
- ** recorded. The uterus of each female that appeared non-gravid

- ** was pressed between two glass slides and examined grossly
- ** for evidence of implantation.
- ** Blood samples were collected at the time of sacrifice and
- ** these were used for a range of serum chemistry
- ** determinations.

**

- ** Each live fetus was weighed and its sex determined and
- ** examined for external anomalies. After gross examination the fetuses were
- * equally distributed into two groups. One group was processed for an
- * evaluation of visceral anomalies and the other group for skeletal
- anomalies.

..

- ** Females in the postnatal groups with surviving offspring
- ** were sacrificed on post partum day 4 and the abdominal
- ** cavity was exposed for a gross examination of the
- ** reproductive organs. The uterus was also examined and then
- ** discarded. All organs were examied macroscopically and the
- ** thymus and liver were weighed.

F008 IUC4

F020 4754

EOR

F002 37

F010 5.8.2

F004 1

F005 RE

F006 Feuston, M. H., Hamilton, C. E., Schreiner, C. A. and

- ** Mackerer, C. R. (1997)
- ** Developmental toxicity of dermally applied crude oils in
- ** rats
- ** J. Tox and Env. Health, Vol 52, pp 79-93

F007 Feuston, M. H., Hamilton, C. E., Schreiner, C. A. and

- ** Mackerer, C. R. (1997)
- ** Developmental toxicity of dermally applied crude oils in
- ** rats
- ** J. Tox and Env. Health, Vol 52, pp 79-93

F008 IUC4

F020 4755

EOR

F002 37

F010 5.8.2

F004 1

F005 RE

F006 Mobil (1991)

- ** Developmental toxicity study in rats exposed dermally to
- ** Belridge heavy
- ** Study No. 63848
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1991)

- ** Developmental toxicity study in rats exposed dermally to
- ** Belridge heavy
- ** Study No. 63848
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F010 5.8.2 F004 1 F005 RM F006 This study was also reported in the open literature by ** Feuston et al. F007 This study was also reported in the open literature by ** Feuston et al. F008 IUC4 F020 4757 EOR F002 37 F010 5.8.2 F004 1 F005 RS F006 Clinical observations ** Treatment related clinical observations in the prenatal and postnatal groups consisted of skin irritation in the 500 ** mg/kg/day group. This included erythema, edema, scabs and open sores at the treatment site. There was F007 Clinical observations ** Treatment related clinical observations in the prenatal and postnatal groups consisted of skin irritation in the 500 mg/kg/day group. This included erythema, edema, scabs and open sores at the treatment site. There was also a red vaginal discharge in animals in this group. One high dose prenatal female had excessive vaginal discharge and, after being found moribund, was sacrificed on gestation day 14. Uterine examination revealed that all but two fetuses had been resorbed. Body weights and food intakes of the 500 mg/kg/day prenatal groups were reduced compared to controls (reduced by approx 31%). Other dose groups were unaffected. Necropsy findings ** With the exception of an approximately 11% increase in ** relative liver weight in the 500 mg/kg/day group, no other ** organ or relative organ weight changes were recorded. [The authors comment that the observed increase in relative liver weight was possibly attributable to the decreased body weight of this group. A prominent vascular pattern of the liver was observed in one female of the 125 mg/kg/day group and two animals in the highest dose group. Serum chemistry The only observation was a 38% reduction of total bilirubin in the prenatal 500 mg/kg/day group. ** Reproductive and fetal evaluations ** Effects only occurred in the highest dose group (500 mg/kg/day). These included

an increase in the mean number/percent resorptions

F020 4756 EOR F002 37

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and 35% compared to 7.8%
                                                respectively) and a corresponding decrease in
                litter size (10.8 compared to 14.5).
**
                A decrease in mean fetal body weights for all
(3.4)
   compared to 3.7).
   Male fetuses in this dose group seemed to be more affected
   than females. Fetal skeletal anomalies recorded were limited to
   incomplete ossification of the nasal bones and caudal centra.
   There were no treatment related visceral anomalies.
   Post partum observations
   During the post partum period there were no clinical
   observations of note except some persistent skin irritation.
   There were no findings at necropsy.
**
   Litter data
** Two females in the 500 mg/kg/day group had no viable
** offspring; their litters were totally resorbed.
** The incidence of pup mortality was two times greater in the
** group treated with crude oil during the lactation period.
** Mean duration of gestation was unaffected, but it was noted
** that the majority of treated females delivered either in the afternoon of
* day 22 or morning of day 23 whereas the
** controls delivered in the morning of day 22. [The biological significance
   of this observation is uncertain.]
F008 IUC4
F020 4758
EOR
F002 37
F010 5.8.2
F004 2
F005 CL
F006 Lost Hills Light crude was shown to be maternally toxic at
** 500 and 2000 mg/kg/day and developmentally toxic at 2000
** mg/kg/day.
F007 Lost Hills Light crude was shown to be maternally toxic at
** 500 and 2000 mg/kg/day and developmentally toxic at 2000
** mg/kg/day.
F008 IUC4
F020 4759
EOR
F002 37
F010 5.8.2
F004 2
F005 ME
F006 Groups of twelve presumed-pregnant Sprague-Dawley rats were
** distributed into six groups as shown below. The test
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** material was applied to the shorn dorsal skin once daily at
** the doses shown from day 0 to day 19 of gestation. The

viable fetuses

** animals were

F007 Groups of twelve presumed-pregnant Sprague-Dawley rats were

- ** distributed into six groups as shown below. The test
- ** material was applied to the shorn dorsal skin once daily at
- ** the doses shown from day 0 to day 19 of gestation. The
- ** animals were shaved weekly and were fitted with collars to
- ** prevent ingestion of the test material.

**

** Group Dose level

** Prenatal groups

** 1 Sham control

** 2 125 mg/kg/day

** 3 500 mg/kg/day

** 4 2000 mg/kg/day

** Postnatal groups

** 5 Sham control ** 6 20000 mg/kg/day

*

- ** Animals were observed at least once daily throughout
- ** gestation until sacrifice for signs of pathosis, abortion,
- ** premature delivery, dystocia and/or death.
- ** Dams and their litters were observed postpartum days 0
- ** through 4. On day 0 postpartum, pups were examined for
- ** external malformations and variations and were observed
- ** daily for the presence of milk in their stomachs.

**

- ** Body weights and food consumption of all prenatal group
- ** females were recorded at regular intervals throughout
- ** gestation. For the post natal groups body weights and food
- ** intakes were recorded throughout gestation
- ** but only body weights were recorded on postpartum days 0 and
- ** 4

*

- ** On day 20 of gestation all prenatal group animals were
- ** sacrificed. The abdominal cavity was exposed and the
- ** reproductive organs examined. Following removal of the
- ** uterus and ovaries the remains were subjected to macroscopic
- ** examination and the liver and thymus were weighed.

**

- ** The uterus and ovaries of each animal were examined grossly.
- ** The number of corpora lutea per ovary was recorded. Ovaries
- ** of non-pregnant females were examined and then discarded.
- ** The uterine contents of each pregnant animal were exposed
- ** and the number and location of all implantations were
- ** recorded. The uterus of each female that appeared non-gravid
- ** was pressed between two glass slides and examined grossly
- ** for evidence of implantation.
- ** Blood samples were collected at the time of sacrifice and
- ** these were used for a range of serum chemistry
- ** determinations.

**

- ** Each live fetus was weighed and its sex determined and
- ** examined for external anomalies. After gross examination the

- ** fetuses were equally distributed into two groups. One group
- ** was processed for an evaluation of visceral anomalies and
- ** the other group for skeletal anomalies.

**

- ** Females in the postnatal groups with surviving offspring
- ** were sacrificed on post partum day 4 and the abdominal
- ** cavity was exposed for a gross examination of the
- ** reproductive organs. After examination, the uterus was
- ** discarded. All organs were examined macroscopically and the
- ** thymus and liver were weighed.

F008 IUC4

F020 4760

EOR

F002 37

F010 5.8.2

F004 2

F005 RE

F006 Feuston, M. H., Hamilton, C. E., Schreiner, C. A. and

- ** Mackerer, C. R. (1997)
- ** Developmental toxicity of dermally applied crude oils in
- ** rats
- ** J. Tox and Env. Health, Vol 52, pp 79-93

F007 Feuston, M. H., Hamilton, C. E., Schreiner, C. A. and

- ** Mackerer, C. R. (1997)
- ** Developmental toxicity of dermally applied crude oils in
- ** rats
- ** J. Tox and Env. Health, Vol 52, pp 79-93

F008 IUC4

F020 4761

EOR

F002 37

F010 5.8.2

F004 2

F005 RE

F006 Mobil (1991)

- ** Developmental toxicity study in rats exposed dermally to
- ** Lost Hills Light
- ** Study No. 63836
- ** Mobil Environmental and Health Science Laboratory

F007 Mobil (1991)

- ** Developmental toxicity study in rats exposed dermally to
- ** Lost Hills Light
- ** Study No. 63836
- ** Mobil Environmental and Health Science Laboratory

F008 IUC4

F020 4762

EOR

F002 37

F010 5.8.2

F004 2

F005 RM

F006 This study was also reported in the open literature by

** Feuston et al.

F007 This study was also reported in the open literature by ** Feuston et al. F008 IUC4 F020 4763 **EOR** F002 37 F010 5.8.2 F004 2 F005 RS F006 There were only few clinical observations that were ** considered to be treatment-related and these were confined to the high dose group (2000 mg/kg/day). The observations consisted of a red vaginal discharge, and one female in this group was F007 There were only few clinical observations that were considered to be treatment-related and these were confined to the high dose group (2000 mg/kg/day). The observations consisted of a red vaginal discharge, and one female in this group was pale in color. Slight skin irritation occurred in a few animals but it is ** not clear whether this was compound-related or self inflicted because the animals attempted to groom themselves, despite the fact that they had been fitted with collars. It ** is possible also that because of this some ingestion of test ** material may have occurred. Animals in the 500 and 2000 mg/kg/day groups gained less weight than the controls over the gestation period (80% and 60% of controls respectively). Food consumption for these two groups was also less than controls for the first 10 days of gestation but was similar to controls thereafter. At necropsy, it was noted that there were more animals in the 2000 mg/kg/day group with a small thymus. Absolute and relative thymus weights were reduced in this group by approximately 50%. A reduction in absolute thymus weight was also recorded for the 500 mg/kg/day group, but the difference was not statistically significant. Liver weights (absolute and relative) were increased in the 500 and 2000 mg/kg group, but only the relative organ weights were statistically significant (11 and 18% increases for the 500 and 2000mg groups respectively). Serum chemistry differences were only observed in the 500 and 2000 mg/kg/day groups These changes were as follows:

**	Parameter	Dose group (mg/k	g/day)	
**		500		2000
**	AST			+27%
**	ALT			+37%
**	Alkaline phosphatase	+	64%	
**	Cholesterol			+22%
**	Triglycerides	-42%		-62%
**	Total bilirubin	-35%		-49%

**	A/G ratio				+15%			
**	Phos[horus				+20%			
**	SDH				+67%			
**								
**	All other ser	rum chemistry measurements v	were unaffected	l by				
**	treatment.							
**								
**	•	ve evaluations						
**	-	ect was a significant increase						
**	•	(6-fold) and a corresponding d	ecrease in litter	•				
**	size (38%) i	n the 200 mg/kg/day group.						
**	-							
**	Fetal evalua			420/ im				
**	Mean fetal body weights were reduced by approximately 13% in the 200 mg/kg/day group.							
**	_	ng/day group. an increase in incomplete ossi	fication of vario	IIS.				
**		ctures which included nasal bo						
**		ases in visceral abnormalities.						
**	The data ar	e shown below:						
**	Date are sh	own as fetal incidence/litter in	cidence					
**								
**	Anomoly			Dose group	(mg/kg/day)			
**			0	125	500	2000		
**								
**	Ossification	variants						
**	nasal bones		1.0/8.3	15b/55	33b/82b	96b/100b		
**	Thoracic ce		4/33	5.5/36	8.5/55	15a/78		
**	Caudal cent		1/8.3	9.9a/36	3.7/36	15b/67b		
**	Sternebrae	<2	2/8.3	6.6/36	7.3/36	23b/67a		
**	\ <i>i</i> ''	16						
**	Visceral ma		0/0		0/0	4.1/22		
**	Right-sided	P<0.05	0/0	-	0/0	4.1/22		
**	a b	P<0.03						
**	b	1 < 0.01						
**	In the prena	atal group, the litter effects wer	·e:					
**	р	ital group, and itale directo ire.	Ο,					
**		Three females had no viable	offspring, their	litters	were			
**	totally resor	bed						
**								
**		Two females had their entire	litters die by		postpartum o	day		
**	3							
**								
**		There was a significant incre	ase in the numb	per of				
**		stillborn pups (7 compared to	2 for controls)					
**								
**		Liveborn pups weighed less t	han the controls	s (-8%		and -		
**	-	s 0 and 4 respectively).						
	08 IUC4							
	20 4764							
EO								
F01	02 37							

F010 5.8.2

```
F004 3
```

F005 ME

F006 Presumed pregnant female Sprague-Dawley rats were used for

** this series of studies.

**

- ** Prudhoe Bay crude oil was administered by gavage according
- ** to the following schedules

** 1. As a single dose (5 ml/kg) on either day 3, 6, 11,

15 or 17 of g

15 or 17 of

F007 Presumed pregnant female Sprague-Dawley rats were used for

** this series of studies.

**

- ** Prudhoe Bay crude oil was administered by gavage according
- ** to the following schedules
- ** 1. As a single dose (5 ml/kg) on either day 3, 6, 11,

* gestation.

** 2. Daily from gestation days 6-17 incl at a dose level

of 1.0 or 2.0

ml/kg.

** 3. As a single dose on day six of gestation at either

2, 5, 7 or 10

* ml/kg.

** Respective controls received equivalent amounts of saline.

**

- ** On day 18 of pregnancy, animals were sacrificed and the
- ** numbers and position of implantations, resorptions and dead
- ** fetuses were recorded. The live fetuses were removed,
- ** weighed and inspected for gross external abnormalities with
- ** the aid of a dissection microscope. Skeletal examinations
- ** and examination of soft internal tissues were not carried
- ** out in this preliminary study.
- ** Student's t-test was used for comparing control and
- ** experimental results. P values were derived from a 2-tailed
- ** table of Student's values for t. The level of significance
- ** chosen was P<0.05.

F008 IUC4

F020 4765

EOR

F002 37

F010 5.8.2

F004 3

F005 RE

F006 Khan, S., Martin, M., Payne, J. F. and Rahimulta, D. (1987)

- ** Embryotoxic evaluation of a Prudhoe Bay crude oil in rats
- ** Toxicology Letters Vol 38, pp 109-114

F007 Khan, S., Martin, M., Payne, J. F. and Rahimulta, D. (1987)

- ** Embryotoxic evaluation of a Prudhoe Bay crude oil in rats
- ** Toxicology Letters Vol 38, pp 109-114

F008 IUC4

F020 4766

EOR

F002 37

F010 5.8.2

F004 3

F005 RL

F006 Probably not conducted to GLP.

- ** Study was a preliminary study and was limited in scope.
- ** Only information on embryo and fetotoxicity was derived in
- ** this study.

F007 Probably not conducted to GLP.

- ** Study was a preliminary study and was limited in scope.
- ** Only information on embryo and fetotoxicity was derived in
- ** this study.

F008 IUC4

F020 4767

FOR

F002 37

F010 5.8.2

F004 3

F005 RS

F006 There were no maternal deaths following oral administration

- ** of PBCO although at high doses the animals exhibited signs
- of intoxication (crouching, indicative of acute irritation).

- Maternal body weight changes were significantly less in
- **

F007 There were no maternal deaths following oral administration

- of PBCO although at high doses the animals exhibited signs
- of intoxication (crouching, indicative of acute irritation).

- Maternal body weight changes were significantly less in
- animals that had been treated with PBCO at early days of
- gestation, in particular those animals given PBCO on day 6
- of gestation only.
- The body weight data are shown in the following tables.
- Note that although the author provided data on mean and
- standard deviation, for simplicity this summary only
- contains mean values (SDs are not shown). Statistically
- significant differences are indicated.

- Body wt changes for dams given a single (5 ml/kg) dose of
- PBCO. Figures in parenthesis indicate group size

*		

**	Treatment	Body weight ch	nange (g on days	indicated)		
**	Day	0-6	6-11	11-15	15-18	0-18
**	Control (11)	30.4	32.6	28	30	121
**	3 (9)	32.75	28.25	23.5*	31	115.22
**	6 (10)	28.8	22.4*	24.2*	27.8	103.2*
**	11 (10)	34.8	35.6	16*	35.2	122.6
**	15 (10)	32.5	33.5	28	35.3	131.5
**	17 (10)	31.67	34	27.33	31.67	124.67
**						
**	*	Significant from	n control (P<0.05	5)		

- ** Effect of different doses of PBCO on dam body weight changes
- ** No. of animals shown in parenthesis

**	Treatment	Body weight change (g on days indicated)					
**		0-6	6-11	11-15	15-18	0-18	
**	Control (8)	27.33	25.67	27.33	32	112.33	

```
Dose (ml/kg) given on day 6 only
   2 (8)
                                                               23*
                                                                              27.5
                                               25
                                                                                              1.3
   5 (10)
                                                                              26.92*
                                                                                              91.22*
                               26.21
                                               18.08*
                                                               19.5*
   7 (8)
                               26.67
                                                                              26.92*
                                                                                              87.67*
                                               17.33*
                                                               15.35*
                               30.67
   10 (8)
                                               14.33*
                                                               11*
                                                                              20*
                                                                                              75.67*
   Dose given on days 6-17 daily
   1 (10)
                                               21*
                                                               23.5*
                                                                              22*
                                                                                              97.5*
   2 (10)
                                                               13*
                                                                              17*
                                                                                              75*
                               27.5
                                               16.5
                               Significant from control (P<0.05)
   The author concluded that the reduced body weight gains were
   probably due to the high resorption rates that occurred.
   Effects on fetal viability and development
   The results of the effect of administering PBCO as a single
   dose of 5 ml/kg at different stages of gestation are
   summarized below:
   Values shown are mean±SD
    significant from control (p<0.05)
   No.
                               Fetal Crown-
           Resorp-
                     No.
   implants tions/
                              weight rump length
                                                                                              per dam dead
      fetuses
                        (mm)
                 (g)
         fetuses
   Day 0
   13.5±2.39 4.68±.93 13±1.24
                                  1.267±.09 22.2±1.44
   14.5±1.92 10.77±1.02* 11.25±1.5 1.19±.08* 20±1.73*
   3.23±1.43 15.48±1.32* 11.02±1.49* 1.2±.09* 1.16±1.38
** Day 11
** 2.9±1.26 8.22±1.08* 11.4±.89* 1.287±.11 21.56±1.37
   Day 15
** 12.72±1.63 5.02±1.19 12.5±.71 1.313±.083 22.65±1.33
   Day 17
   13±1.8
            3.62±.89 12.33±1.15 1.304±.124 23.01±1.41
** These results demonstrate that the incidence of resorptions
   and dead fetuses was increased in the animals given PBCO
   during the early days of pregnancy whereas those given PBCO
   during the later stages of pregnancy were unaffected. The
   greatest effect was on day 6 of gestation.
   Repeated daily administration of low doses (1 or 2 ml/kg) on days 6
   through 7 showed an additive effect and at a dose
   level of 2 ml/kg/day resulted in up to 40% fetal deaths.
   These data are shown in the following table.
```

```
**
                The effect of different doses of PBCO
   Values shown are mean ± SD
    * significant from control (p<0.05)
**
              Resorp-
                        No.
                                 Fetal Crown-
      implants tions/
                         live
                                 weight rump length
      per dam dead
                         fetuses
                                           (mm)
            fetuses
             (%)
   Dose (ml/kg)
   0 13.95±2.23 3.96±0.87 13.72±1.56 1.309±.091 21.62±1.17
** Day 6
** 2 14±2.63 6.84±1.21 13.85±2.33 1.326±.119 21.78±1.54
** 5 13.26±1.95 12.67±1.18* 11.73±1.47* 1.216±.09* 21.54±1.27
** 7 12.93±2.35 13.23±1.35* 11.68±1.46* 1.2±.109* 21.28±.89
   10 14.12±2.12 22.86±1.98* 10.5±1.59* 1.192±.108* 0.73±1.19
**
** Day 6-17 (daily)
** 1 13.92±2.46 17.5±2.02* 10±1.95* 1.184±.107 0.16±1.34*
** 2 12.93±1.98 43.8±1.92* 8.72±1.34* 1.182±.097* .96±1.31*
   In conclusion, administration of PBCO to pregnant females
   resulted in an increased incidence of resorptions, increased fetal death
   and decreased fetal body weight. These effects occurred at doses which
   were maternally toxic.
F008 IUC4
F020 4768
FOR
F002 37
F010 5.8.3
F004 1
F005 ME
F006 Groups of five 10-12 week old male mice were injected i.p.
** daily for 5 days with test samples in trioctanoin at doses
** of 1 and 2.1 g/kg. Control mice were injected with
** trioctanoin (5x0.25 ml) or BaP (5x0.05 or 1x0.07g).
** After 35 days, the
F007 Groups of five 10-12 week old male mice were injected i.p.
** daily for 5 days with test samples in trioctanoin at doses
** of 1 and 2.1 g/kg. Control mice were injected with
** trioctanoin (5x0.25 ml) or BaP (5x0.05 or 1x0.07g).
** After 35 days, the animals were sacrificed, the cauda
** epididymes were removed, combined and minced in 0.9% NaCl or
** Tyrode's solution and the sperm were stained by adding eosin
** Y solution. The preparations were spread on slides and
** air-dried. The percentage of abnormal sperm was determined
** in 500 sperm from each animal. For control of bias in
** scoring, positive and negative control slides from a
   previous experiment were included at a ratio of 1 bias
** control slide to 5 new slides.
```

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F008 IUC4
F020 4769
EOR
F002 37
F010 5.8.3
F004 1
F005 RE
F006 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and
** Sabharwal. P. S. (1982)
** Comparative study of the genotoxic properties of Eastern and
** Western U. S. shale oils, crude petroleum and coal-derived
** Mutation Research Vol 102, pp
F007 Lockard, J. M., Prater, J. W., Viau, C. J., Enoch, H. G. and
** Sabharwal. P. S. (1982)
** Comparative study of the genotoxic properties of Eastern and
** Western U. S. shale oils, crude petroleum and coal-derived
** Mutation Research Vol 102, pp 221-235
F008 IUC4
F009 28-08-2003
F020 4770
EOR
F002 37
F010 5.8.3
F004 1
F005 RS
F006 The data from the study are given in the following table and
   show that treatment with Wilmington crud did not cause an
   increase in the percentage of abnormal sperm.
                                                                               Abnormal sperm
   Sample
               Dose
                                               No. survivors/
                                               no. treated
                                                               (%±SD)
**
   Triocta
F007 The data from the study are given in the following table and
   show that treatment with Wilmington crud did not cause an
   increase in the percentage of abnormal sperm.
                                               No. survivors/
               Dose
                                                                               Abnormal sperm
   Sample
                                               no. treated
                                                               (%±SD)
   Trioctanoin
               5x0.25 ml
                               10/10
                                                               4.5±1.7
   Wilmington crude
               5x2.1 g/kg
                               5/5
                                                               6.1±2.1
               5x1.0 g/kg
                               5/5
                                                               5.4±1.3
** BaP
               5x0.05 g/kg
                                                               19.7±16.6
                               10/10
```

```
F020 4771
EOR
F002 37
F010 5.9
F004 1
F005 ME
F006 The authors summarized the protocol in the following table.
   Animals
   Charles River (ChR-CD) male mice
   Six weeks old
** Toe clipped for identification
   Backs shaved free of hair
   Group size
   30 mice per group
   Housing
   2 mice per cage
   Diet
   Water
F007 The authors summarized the protocol in the following table.
   Animals
   Charles River (ChR-CD) male mice
   Six weeks old
   Toe clipped for identification
   Backs shaved free of hair
   Group size
   30 mice per group
   Housing
   2 mice per cage
   Diet
   Water and laboratory chow ad libitum
   Observation
   Clipping of hair on the back
   Weekly, as needed
   Treatments
   Initiation:
                               50µl material as received
   Promotion:
                               2 weeks after initiation treatment
   2.5µg phorbolmyristate acetate (PMA)/0.1ml acetone
   Three times a week for 180 days
   Parameters measured
```

Body weight, recorded weekly

```
** Time to development of first tumor
** Tumors charted monthly
** Mortality
F008 IUC4
F020 4772
EOR
F002 37
F010 5.9
F004 1
F005 RE
F006 Calkins, W. H., Deye, J. F., Hartgrove, R. W., King, C. F.
** and Krahn, D. F. (1981)
** Synthetic crude oils from coal: Mutagenicity and
** tumor-initiating screening tests.
** In Mahlum, D. D. et al (Eds)
** Coal conversion and the environment pp 461-47
F007 Calkins, W. H., Deye, J. F., Hartgrove, R. W., King, C. F.
** and Krahn, D. F. (1981)
** Synthetic crude oils from coal: Mutagenicity and
** tumor-initiating screening tests.
** In Mahlum, D. D. et al (Eds)
** Coal conversion and the environment pp 461-470
** U. S. Dept. of Energy, Washington D. C.
F008 IUC4
F020 4773
EOR
F002 37
F010 5.9
F004 1
F005 RL
F006 The study was a preliminary screen and was not fully
** reported. The data are however are useful in assessing the
** tumor initiating activity of crude oil.
F007 The study was a preliminary screen and was not fully
** reported. The data are however are useful in assessing the
** tumor initiating activity of crude oil.
F008 IUC4
F020 4774
EOR
F002 37
F010 5.9
F004 1
F005 RS
F006 Sample
               No. mice
                                Av No.
                                                               No. days
                with
                                                tumors/
                                                                               to first
                                               tumor-
               tumors
                                                                               mouse with
               lasting
                                               bearing
                                                                               tumor
               >30 days
                                mouse
   Benzo[a]pyrene positive control
                                               6.0
                                                                               48
```

** South Louisiana Crude

**	5		1.4	71
** Tolue	ne and PMA			
**	4		1.5	111
** PMA				
F007 Sam	nple No. mice	Av No.	N	o. days
**	with		tumors/	to first
**	tumors		tumor-	mouse with
**	lasting		bearing	tumor
**	>30 days	mouse		
**				
** Benzo	[a]pyrene positive	control		
**	26		6.0	48
** South	Louisiana Crude			
**	5		1.4	71
** Tolue	ne and PMA			
**	4		1.5	111
** PMA a	alone			
**	3		1.0	92
**				

 ** The authors concluded that the South Louisiana crude oil was

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^{**} not an initiator.